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PULMONARY CHANGES FOLLOWING EXPOSURE TO PHOSGENE *

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The pathologic changes in the respiratory tract following exposure to phosgene have been described by Winternitz [1] and Vedder.[2] No mention was made of pulmonary consolidation such as is described in this report. This was encountered during studies of the therapeutic effect of 60 per cent oxygen upon dogs surviving exposure to phosgene. It was responsible for the late deaths from anoxia occurring after recovery from the earlier anoxia associated with pulmonary edema.

METHODS

Dogs were observed for 3 to 4 days prior to gassing and only such animals as appeared healthy were utilized. Exposure to phosgene was maintained for 30 minutes in a dynamic chamber at an average concentration of 0.29 mg. per L. After exposure animals were treated according to the following procedures:

A. No treatment.

B. 10 cc. per kg. of blood removed after exposure.

E. Small amounts of plasma administered intravenously as indicated by fall in blood pressure or tachycardia.

F. Maintained in an atmosphere of 60 per cent oxygen when the arterial oxygen saturation was below 80 per cent. Plasma as in group E.

G. Oxygen treatment as in group F. No plasma.

H. Maintained in 60 per cent oxygen.

The dogs used in this study have been divided according to survival after exposure, into four groups, as defined subsequently.

At autopsy an incision was made in the neck and a clamp placed firmly about the trachea to prevent escape of fluid from the respiratory

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tree. The ratio of lung to body weight was determined; a ratio of 0.015 was found to be the upper limit of normal. Tissue for microscopic section was fixed in 10 per cent neutral formalin. Paraffin sections stained with hematoxylin and eosin, Masson's stain for connective tissue, Weigert's stain for elastic fibers, and phosphotungstic acid-hematoxylin stain for fibrin were used.

Results

Summary of Alterations in Oxygenation of Blood Following Exposure to Phosgene. Immediately after exposure to phosgene, dogs showed a transient fall in the oxygen saturation of the arterial blood, due presumably to bronchiolar constriction. Several hours later there was a progressive, more profound anoxemia caused by pulmonary edema; death often occurred during the first 48 hours after exposure. The oxygen saturation of surviving animals gradually returned to normal. Administration of 60 per cent oxygen to dogs during the stage of pulmonary edema caused a temporary rise in arterial oxygen saturation. and reduced the number of deaths. However, a later phase of anoxemia 2½ to 4 days after exposure was observed in a few untreated animals that had survived the period of pulmonary edema. It was the rule in comparable animals that had received oxygen. This late anoxemia accounted for the fact that the mortality rate after oxygen was not significantly lower than for untreated animals.[3]

Anatomical Changes Following Phosgene. The essential pulmonary findings in the dogs comprising this report are outlined in Table I.

Group I. Seven Dogs Surviving Not More Than 3 Days after Gassing

Gross Findings. The lungs were voluminous and heavy. Below the smooth, moist, transparent pleural surface there were alternate soft, pale pink, emphysematous areas and dark red, depressed, firm, noncrepitant, edematous areas which pitted on pressure. When sectioned, considerable quantities of pale yellow, occasionally blood-tinged fluid and froth poured from the tissue and bronchi. The loose peribronchiolar tissue and the subpleural and interlobular lymphatics were distended by fluid. The major bronchi were filled with frothy fluid; their mucosal surfaces were smooth and pale.

Microscopic Findings. The serosal surface of the pleura was free of exudate. Patches of enlarged, air-containing, thin-walled alveoli were found interposed between zones of smaller alveoli filled with pink-staining, protein-rich fluid and strands of fibrin; rare polymorphonuclear leukocytes, small numbers of red blood cells, and occasional mononuclear cells were present in the exudate. The capil-

Summary of Anatomical Findings after Exposure to Phosgene

Gross findings	Alveolar exudate	Alveolar walls	Bronchioles

Summary of Anatomical Findings after Exposure to Phosgene

Dog. no.	Time post-gassing	Gross findings					Alveolar exudate						Alveolar walls			Bronchioles			
		Lung/body ratio	Edema	Emphysema	Atelectasis	Consolidation	Edema	Fibrin	Red blood cells	Polymorphonuclear cells	Histiocytes	Organization	Congestion	Cellular exudate	Scars	Mucosal necrosis	Cellular exudate	Organization	Scars
Group I																			
1A*J	16 hrs.	0.029	+++	+++	++		+++	++	+	+			+++			++			
2F	35 hrs.	0.059	++++	++	+		+++	+++	+	+			++			+++			
3F	40 hrs.	0.042	+++	+	++		+	++	+	++			+			+++	++		
4H	42 hrs.	0.049	++	+++	++		+	+	+	+			+			++			
5B	42 hrs.	0.034	+++	++	+++		+++	++		+			+	+		+++	++	+	
6G	60 hrs.	0.036	++++	++	++		++++	+	++	++++	+		++			++	++++		
7H	72 hrs.	0.029	++	+	+		++	++	+	+	+		+	+		++		+	
Group II																			
8G	4 days	0.027	+	++		++	+	++	+	+	++			++		+	++	+	
9F	4 days	0.048	++	+		++	+	++	+		++	+		+		+	+	+	
10H	4 days	0.019	+	+++	+		+	+			+			+				+++	
11G	4½ days	0.021			++	++	+	+			+	++		++				+++	
12H	4½ days	0.035	+	+	+	+++	+	+	++	+	++	+++		++		+	++	+++	
13G	4½ days	0.030			+	++	+	+	++		+++	++++		+++		+		+	
14H	4½ days	0.016	+	+	+	+			+		+	+++		++			++	++	
15E	4½ days	0.016	++		+	++	++	+			+	+++		+++			+	+++	
16H	5 days	0.014	+	++			+		+		+	+		++				++	
17F	5 days	0.026				+++					++++	+++		+++			++	++++	
18H	5½ days	0.034	+	+	+	+++	+	++	++		+++	+++	+	++			++	+	
19A	6 days	0.026				+++		+	+	+	+	+++		+++			+	+	
20H	7 days	0.015	+	+	+	+				++++	+	+		+		+	++++		
21H	7 days	0.025				+++			+		+	+++		+++				+++	
22H	8 days	0.032		++	+	+++				+	+++	+++		+++		+	++	++	
23H	8 days	0.033	+	+	+	+++	+	+	+		+	+++		+++	+			+	
24H	9 days	0.014										+		+++				+	
25E	9 days	0.023		+		+++						++++		++++				+++	++
Group III																			
26G	27 days	0.016		+	++									+++	++				+
27G	59 days	0.013		++	++									++	++				++
Group IV																			
28		0.010																	
29		0.015												+					
30		0.015		+															
31		0.017		+															

* The letters indicate the procedure employed following gassing.

laries of the alveolar walls were congested but no thrombi were noted. Necrosis of alveolar structures was not observed, but there was necrosis of the mucosa of small bronchioles with plugs of desquamated epithelial cells, fibrin, and necrotic débris within the lumina. Metaplastic changes of the regenerating bronchiolar epithelium were found in those dogs that survived longest in this group. The major bronchi and blood vessels showed no changes. In dog 6 purulent bronchitis and focal pneumonia were found.

Group II. Eighteen Dogs Surviving 4 to 9 Days after Gassing Irrespective of Treatment

Gross Findings. The pleural surface was transparent and free of exudate. The lungs showed emphysema and edema but these were not so extensive as in the early post-gassing period. In the majority of instances there was consolidation of one or more lobes with firm pale parenchyma of rubbery consistency (Fig. 1). The cut surface of these lobes was dry, gray, and granular, and bulged above the pleura (Fig. 2). The bronchi gaped widely and contained mucoid material. In the non-consolidated regions small, raised, gray, glistening, tubercle-like lesions were observed, apparently surrounding small bronchioles. No changes were noted in the major blood vessels.

Microscopic Findings. There was no exudate on the serosa of the pleura. During the fourth and fifth days after gassing, small amounts of fluid, fibrin, red blood cells, and remnants of polymorphonuclear leukocytes persisted in the alveoli. In most places this material had coagulated into an amorphous, deep-red-staining mass. The alveolar lining cells were swollen and obscured the alveolar walls. Later, the alveolar walls were infiltrated and the alveoli filled with large macrophages having round or oval, vesicular nuclei with the chromatin distributed about the periphery and a prominent, centrally placed nucleolus (Fig. 3). The involved parenchyma was diffusely consolidated and the architecture ill defined. At this stage, the bronchioles were the only structures in which organization could be seen (Fig. 4). In those animals surviving longer, well formed fibroblasts were present within the alveoli (Fig. 5), and collagen was detectable by the Masson technic. Where alveolar walls could be clearly identified by elastic tissue stain, it could be observed that organization did not arise from intact alveolar walls, but appeared to extend into the alveoli from points of attachment in the bronchioles. At this stage the involvement of the lobes was not diffuse. Organization of alveolar exudate was confined to focal areas with a peribronchiolar distribution; the intervening alveoli were air-containing and their walls thick and cellular. Plugs of tissue of organization, covered by proliferating islands of cuboidal or squamous

epithelium, entirely or partly occluded the bronchioles in all animals except dog 20 (Fig. 6). This animal had a suppurative bronchiolitis and focal pneumonia unaccompanied by the proliferative processes described above.

Group III. Two Dogs That Had Shown Anoxia 4 to 9 Days after Exposure but Had Recovered

Gross Findings. There were no pleural adhesions. Patchy emphysema and small foci of atelectasis were found beneath the smooth, glistening pleura. The pulmonary parenchyma was crepitant, but an increased resistance with a rubbery consistency, particularly of the lower lobes, was noted. When sectioned, the parenchyma presented a dry, spongy, pink surface. In the subpleural region on the posterior surfaces of the lower lobes in dogs 26 and 27 there was a thin band of firm, white, noncrepitant tissue. No changes were observed in the major bronchi or blood vessels.

Microscopic Findings. The alveoli were free of exudate. Thickening of the alveolar walls by collagen and mature fibrocytes was present in a few focal zones and also diffusely in other areas. Many bronchioles were partially or completely obstructed by fibrous connective tissue plugs, the lumina frequently showing "recanalization" around the plug with re-epithelialization of the air passages (Fig. 7). In dogs 26 and 27 there was atelectasis of the subpleural parenchyma in the posterior portions of the lower lobes where scarring of the bronchioles was so marked as to result in complete obstruction (Fig. 8). A few small adventitial hemorrhages were present about the larger pulmonary vasa.

Group IV. Four Dogs Not Exposed to Phosgene but Maintained Continuously in an Atmosphere of 60 Per Cent Oxygen for 7 Days before They Were Examined

Gross Findings. There were no significant gross changes. A few small emphysematous blebs were present in the anterior margins of the upper lobes.

Microscopic Findings. The alveolar walls were intact and the lumina free of exudate. The bronchial tree was lined by well preserved mucosa and showed no change. A few small foci of emphysema and atelectasis were observed.

PROTOCOLS OF REPRESENTATIVE ANIMALS FROM GROUPS II AND III

Dog 17, Group II

Mongrel, male, weight 15.4 kg. Maximum hemoconcentration of 46 per cent was attained 17 hours post-gassing, at which time an arterial oxygen saturation of less than 80 per cent was first observed. The ani-

mal was placed in an atmosphere of 60 per cent oxygen 3 hours later, when the oxygen saturation of the arterial blood was 70 per cent. A minimum arterial oxygen saturation of 68 per cent was observed 28 hours post-gassing, while the animal was breathing 60 per cent oxygen. Plasma in the amount of 2.6 cc. per kg. was administered in two doses 15 hours post-gassing. Oxygen therapy was continued for 54 hours; at this time the arterial oxygen had returned to 88 per cent saturation. A fall in saturation to 57 per cent was observed when the animal was removed from the oxygen chamber, 3 days post-gassing. There was subsequent recovery in room air to 85 per cent saturation 5 days post-gassing.

Gross Findings. The lungs weighed 400 gm. There was emphysema and congestion of the upper lobes bilaterally. A few small foci of depressed, noncrepitant pulmonary parenchyma of moist consistency were also noted. The middle and lower lobes were completely consolidated, noncrepitant, and rubbery. On cut section their parenchyma bulged above the pleura and was granular and dry.

Microscopic Findings. The pleura was thin and the parenchyma below it completely without air-containing tissue. The structure of the alveolar walls was not recognizable, and the tissue within the alveoli consisted of a solid mass of young proliferating fibroblasts and large mononuclear phagocytes. Many of these mononuclear cells showed mitotic figures. The terminal bronchioles were filled with plugs of proliferating epithelium of squamous and cuboidal type, supported and nourished by a stroma of young capillaries and fibroblasts. The mucosa of the smaller bronchi showed extensive metaplastic change. The entire bronchus in places was lined by a layer of squamous epithelium three or four cells deep. The larger bronchi, on the other hand, showed no mucosal changes. The submucosa was free of exudate and the lumina contained a few polymorphonuclear leukocytes and pink amorphous substrate. The walls of the blood vessels showed no changes. There was no evidence of thrombosis or ring hemorrhage.

Dog 23, Group II

Mongrel, female, weight 11.8 kg. The animal was placed in a chamber containing 60 per cent oxygen 25 minutes post-gassing and a maximum hemoconcentration of 30 per cent was attained in 19 hours. A minimum arterial oxygen saturation of 49 per cent was observed 30 hours after exposure to phosgene with subsequent recovery to 70 per cent 55 hours later. Four days after gassing, the arterial oxygen saturation was 64 per cent and there was a further progressive decline until death 8½ days post-gassing. The arterial oxygen saturation was 35

per cent 3 hours before death. The animal had remained in 60 per cent oxygen uninterruptedly until death.

Gross Findings. The lungs weighed 390 gm. The pleural surfaces were smooth and glistening. There were blebs of emphysema in the upper lobes, particularly along the anterior margins. Severe emphysema was noted in the post-cardiac and middle lobes. There was dilatation of the subpleural lymphatics, particularly over the lower lobes. Both lower lobes were firm and noncrepitant. They maintained their shape, and on cut section firm, rubbery tissue bulged above the pleura. The parenchyma was pale, gray, granular, and opaque. The bronchi and bronchioles gaped widely and contained a small amount of viscid material. There was no pus within the bronchi and no fluid or exudate could be scraped from the surface of the lower lobes. The upper lobes were moist on cut section and showed alternate zones of congestion and emphysema.

Microscopic Findings. Throughout all sections the alveoli were filled with large cells containing vesicular oval nuclei and abundant, blue-staining cytoplasm which gave off fibrillary processes. When stained by the Masson technic, these processes took up the green dye. The alveolar walls were preserved throughout all lobes and the young tissue of organization within the alveoli did not appear to be attached to the alveolar walls. The bronchioles showed plugs of young tissue which filled the lumina. In the upper lobes the organization within the alveoli was not as diffuse and alternate zones of emphysema, atelectasis, and hemorrhage were noted in the parenchyma.

Dog 25, Group II

Mongrel, female, weight 15.7 kg. Maximum hemoconcentration of 37 per cent above immediate post-gassing value was attained 14 hours after exposure to phosgene. At this time an arterial oxygen saturation of less than 80 per cent was first observed; progressive fall continued until 48 hours post-gassing, when a minimum saturation of 49 per cent was attained. Two courses of plasma totaling 5.0 cc. per kg. were administered 14 and 20 hours post-gassing, respectively. The arterial oxygen saturation remained below 80 per cent for a total of 7 days, returning to 84 per cent on the eighth day. It was 83 per cent the following day, when the animal was sacrificed with sodium pentobarbital intravenously.

Gross Findings. The lungs weighed 355 gm. They were heavy and of a rubbery consistency throughout. In the upper lobes there was some crepitation but the lower lobes appeared to be entirely solid. Small zones of marginal emphysema were apparent in the upper lobe.

On cut section the lower lobes were airless and showed an unusually pale, granular, firm, dry surface. The upper lobes were pale and had an increased resiliency.

Microscopic Findings. The alveolar spaces were filled with fibroblasts and there was well formed collagen within the alveolar spaces. The alveolar walls were preserved, as could be recognized in sections stained for elastic fibers. There was no other cellular exudate in any of the alveoli and there was no evidence of edema or hemorrhage in either upper or lower lobes. In the upper lobes the distribution of connective tissue in the alveoli was patchy, whereas in the lower lobes it was diffuse and confluent. The smaller bronchioles were plugged with masses of tissue of organization. The large bronchi and blood vessels were free of change.

Dog 27, Group III

Mongrel, male, weight 12.5 kg. Maximum hemoconcentration of 40 per cent was attained 12 hours post-gassing, at which time an arterial oxygen saturation of less than 80 per cent was first observed (77 per cent). The animal was placed in an atmosphere of 60 per cent oxygen 1 hour later. A minimum arterial oxygen saturation of 63 per cent was observed 40 hours post-gassing, while the animal was breathing 60 per cent oxygen. Four days post-gassing the oxygen saturation had risen to 82 per cent, but thereafter slowly fell to another point of minimum saturation (69 per cent) 8 days post-gassing. Gradual recovery was resumed and the arterial oxygen saturation was 92 per cent just before removal of the animal from the oxygen chamber 17 days post-gassing. It was sacrificed with sodium pentobarbital intravenously 50 days post-gassing; the arterial oxygen saturation was 91 per cent at that time. Momentary removal from the oxygen chamber at 3 and 7 days post-gassing was followed by prompt collapse and respiratory arrest, with as rapid recovery when the animal was returned to an atmosphere of 60 per cent oxygen and given artificial respiration. The body weight fell to 8.9 kg. 19 days post-gassing and was 9.4 kg. at the time of death, although the dog appeared healthy otherwise and was eating well.

Gross Findings. The pleura was thin and transparent except over the posterior surfaces of both lower lobes, where a zone of thick, white, firm, opaque tissue, 0.5 cm. in thickness, was present in the subpleural region. The remaining lung was pink and crepitant but had an increased resiliency throughout. There were a few small, depressed, dark red, noncrepitant, atelectatic zones in the post-cardiac and right lower lobe. The major bronchi and blood vessels showed no changes.

Microscopic Findings. The pleura was thin, and below it, in the post-cardiac lobe and in both lower lobes, were thin zones of atelectatic pul-

monary parenchyma. These zones were associated with small bronchioles which were completely obstructed by dense fibrous connective tissue plugs. In some areas there was marked emphysema. Elsewhere the alveolar walls were well preserved but were thickened by collagen fibers and numerous fibrocytes. The bronchioles throughout the sections showed complete or partial obstruction of their lumina by plugs of scar tissue, the surfaces of which were covered by columnar epithelium. The major bronchi were well preserved, as were the blood vessels. The blood vessels showed some edema of the adventitia, but no other change.

Discussion

While proliferation of fibroblasts with organization of exudate in and about small bronchioles has been reported in dogs following exposure to phosgene, the extension of this process to involve an entire pulmonary lobe has not hitherto been encountered.[1] The development of lobar consolidation is dependent upon two factors: severe pulmonary injury due to phosgene, and a survival period adequate for the development of the lesion.

The dogs of group II with severe pulmonary injury due to phosgene survived the phase of pulmonary edema because of oxygen therapy. They subsequently developed anoxia, not relieved by oxygen, that resulted from extensive pulmonary consolidation. That this organization was not a consequence of oxygen therapy is indicated by the occurrence of similar lesions in 3 animals not given oxygen after exposure, and by the absence of pulmonary changes in control animals exposed to oxygen alone for a comparable period.

The paucity of polymorphonuclear leukocytes in the exudate of the majority of instances indicates that the consolidation was not the result of an infectious process. This is borne out by the absence of organisms in aerobic cultures of the lungs of 3 animals. Dog 20 had a purulent pneumonia presumably of bacterial origin and differed from the others of this group, in which histiocytic and fibroblastic proliferation predominated.

Microscopic study of lungs stained with Weigert's elastic tissue stain indicated that destruction of the alveolar walls had not occurred in the consolidated lobes. The granulation tissue within the alveoli did not appear to originate from the alveolar walls but rather to extend into the adjacent alveoli from points of attachment within a terminal bronchiole. These observations explain why so few parenchymal scars were observed in the 2 dogs of group III which survived an episode of anoxia during the period of 2½ to 4½ days after gassing. The discrepancy between the amount of exudate at the stage when organization had

begun and the amount of ultimate scar tissue indicates that more of the exudate had resolved than would have been anticipated. This may be explained by the fact that only minor alveolar damage is caused by phosgene and by the absence of persistent chemical or bacterial irritating agents within the alveoli. The only large scars in the lungs of these animals were found in the bronchiolar lumina. It is noteworthy that in both of these dogs there were a few areas where the alveolar walls were slightly thickened by mature collagen fibers. Such interstitial scarring might be interpreted as reflecting earlier damage to the alveolar walls by phosgene.

Summary and Conclusions

1. Consolidation of one or more lobes of the lung has been found in dogs 4 to 9 days after exposure to phosgene (0.29 mg. per L. for 30 minutes). In addition to "obliterative bronchiolitis," the involved lobes showed a diffuse mononuclear exudate within the alveoli and foci of thickening and organization of the alveolar walls composed of large mononuclear cells and young fibroblasts.

2. Pulmonary organization occurred as the initial edema subsided. This resulted in severe late anoxemia and caused high mortality in spite of oxygen therapy during the period.

3. Two dogs that had survived the stage of pulmonary organization, as indicated by "clinical" observation, showed only focal scars in the pulmonary parenchyma and bronchioles 27 and 59 days after gassing.

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2. Vedder, E. B. The Medical Aspects of Chemical Warfare. Williams & Wilkins Co., Baltimore, 1925.
3. Ordway, N. K., Harrison, H. E., Bunting, H., and Albrink, W. S. Unpublished data.

DESCRIPTION OF PLATES

Plate 114

Fig. 1. Dog 23 (8 days). Consolidation of all lobes with subpleural emphysema.

Fig. 2. Dog 23 (8 days). Cut section of right lower lobe, showing the solid character of the parenchyma.

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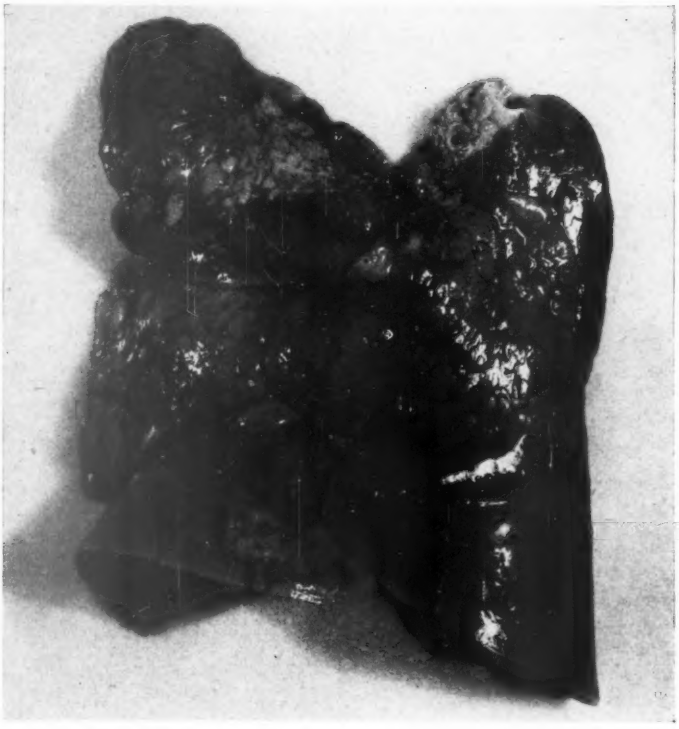

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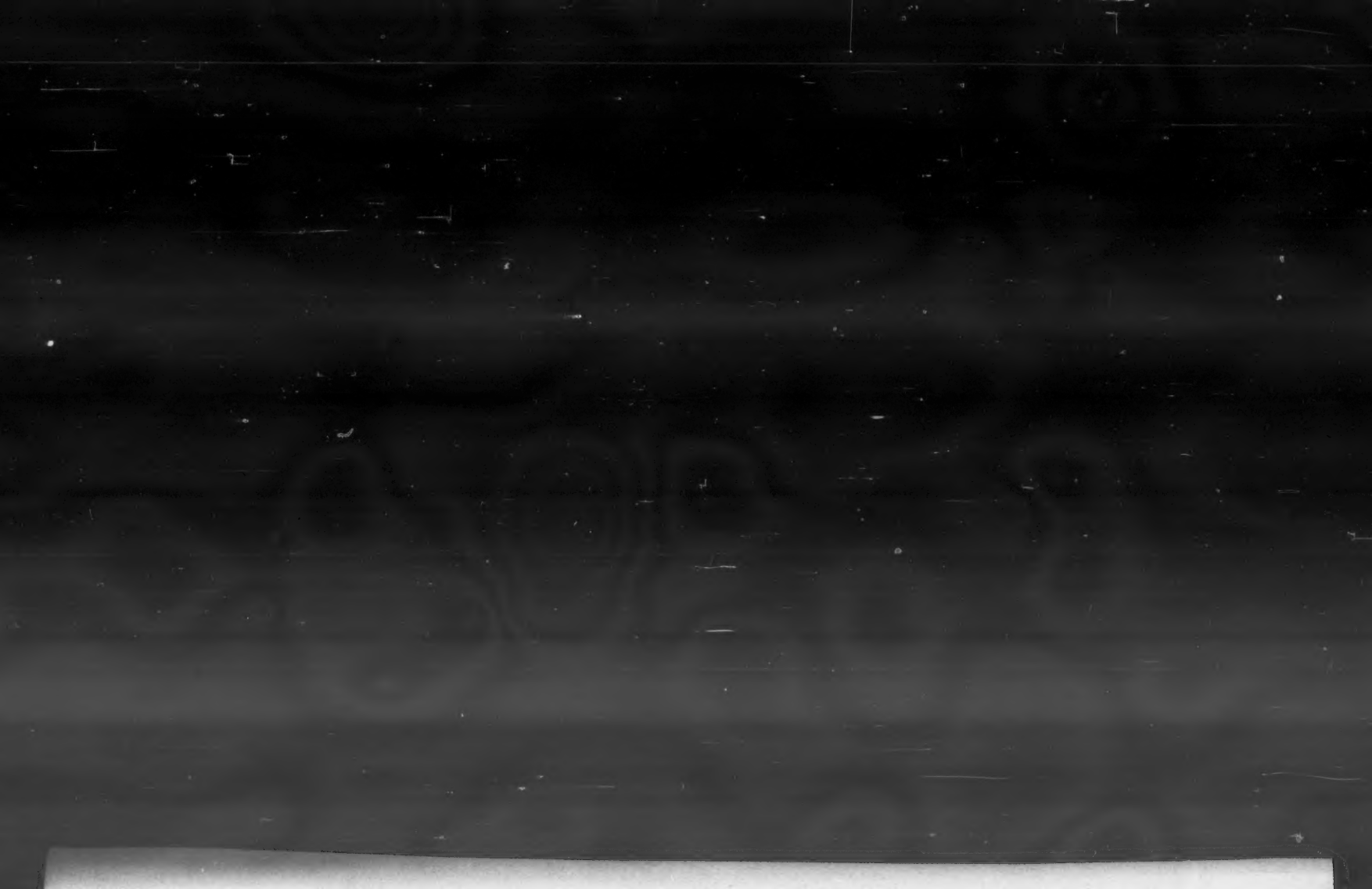
PLATE 115

FIG. 3. Dog 23 (8 days). Large mononuclear cells and polymorphonuclear leukocytes in alveoli. × 300.

FIG. 4. Dog 17 (5 days). Plug of newly formed tissue in bronchus with metaplasia of lining epithelium. × 105.

FIG. 5. Dog 25 (9 days). Plugs of organizing tissue in alveoli. × 300.



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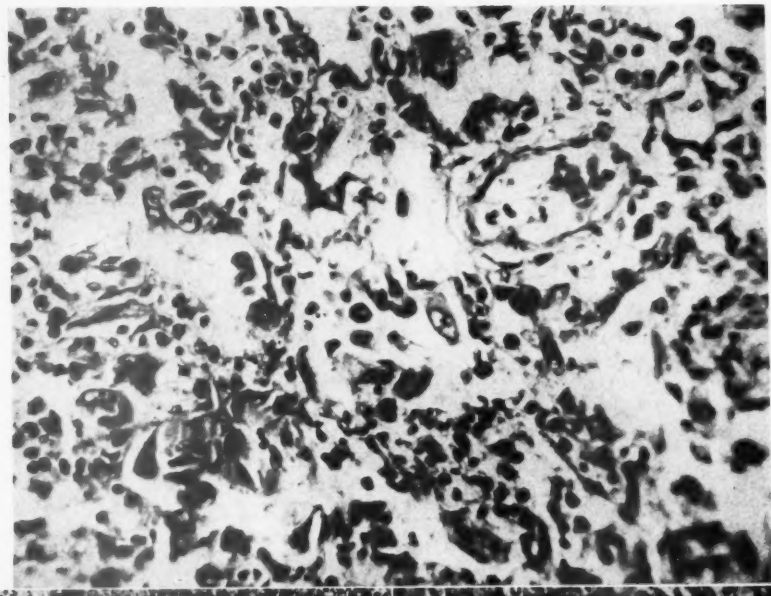

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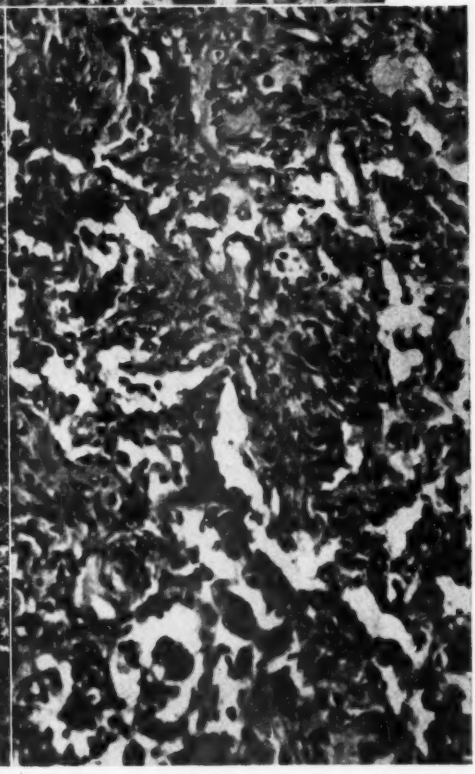

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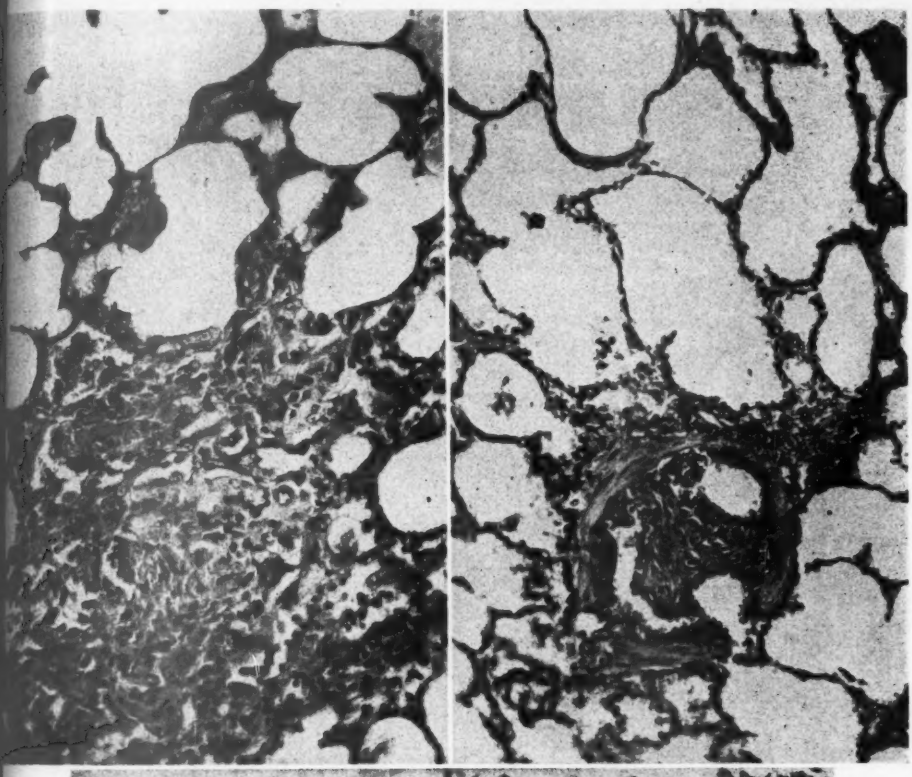
Durlacher and Bunting Pulmonary Changes Following Phosgene

PLATE 116

FIG. 6. Dog 21 (7 days). Bronchiole filled with fibroblastic tissue and proliferating epithelial cells. Thickening of walls of surrounding alveoli. × 220.

FIG. 7. Dog 27 (59 days). Re-epithelialized scar in bronchiole. × 100.

FIG. 8. Dog 27 (59 days). Subpleural atelectasis and scarring. Obliteration of bronchioles by scar tissue. × 45.



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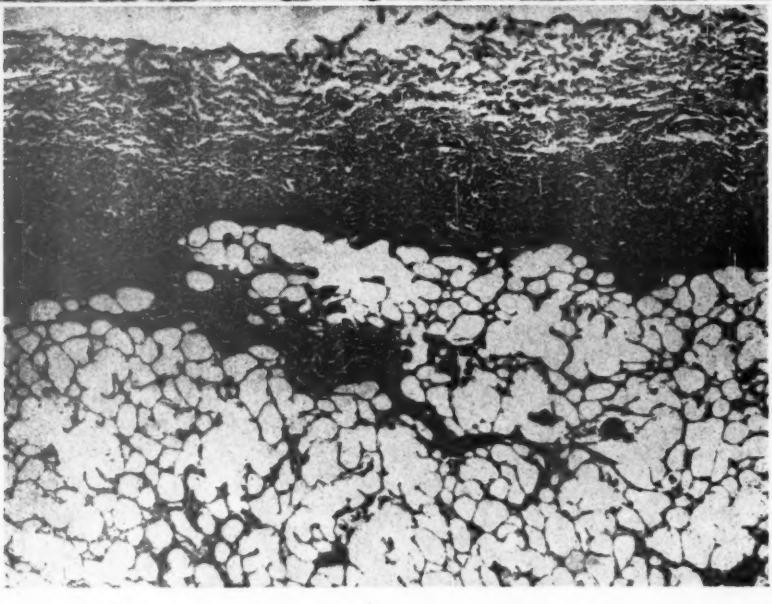

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Durlacher and Bunting Pulmonary Changes Following Phosgene

STUDIES OF THERMAL INJURY

II. The Relative Importance of Time and Surface Temperature in the Causation of Cutaneous Burns *

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Although it is common knowledge that there is an inverse relationship between the intensity of a thermal exposure and the amount of time required to produce a burn, there is remarkably little available information as to the rate at which burning of human skin occurs at any given surface temperature or as to the pathogenesis and pathological characteristics of burns in which the duration and degree of rise in intracutaneous temperature was known or could be calculated with any degree of accuracy.

Considerable information regarding the time-temperature thresholds at which cutaneous burning occurs in animals is provided by the experiments of Hudack and McMaster [1] and of Leach, Peters, and Rossiter.[2] In the former, water at temperatures ranging between 42° and 67°C. either was applied directly or was passed through a thin-walled glass chamber, the base of which was brought in contact with the skin of mice. In the experiments performed by Leach, Peters, and Rossiter water was pumped through a metal chamber at temperatures ranging between 45° and 80°C. and the base of the chamber was held in contact with the skin of guinea-pigs for varying periods of time. Both groups of investigators observed that the time required to produce injury diminished rapidly as the temperature of the water was raised. The former reported that a source temperature of 44°C. was critical for the causation of hyperthermic edema. The latter reported that the critical temperature for causing permanent and irreversible injury of guinea-pig skin lies between 50° and 55°C. Neither of the above-cited investigations provided data from which the time-temperature requirements for the production of burns of human skin could be estimated.

Although Leach, Peters, and Rossiter [2] made a careful study of the pathological characteristics of different kinds of burns of guinea-pig skin, the extent to which these changes are representative of those that occur in cutaneous burning in man was not disclosed.

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The primary purpose of this investigation was to obtain information regarding the effects on human skin of episodes of hyperthermia of varying duration and of varying degrees of intensity. The direct approach would have been to make all experiments on human subjects. For various reasons this was not feasible. It was decided first to establish the time-temperature thresholds for varying degrees of cutaneous injury by experiments on an animal having a skin similar to that of man, and then by means of a relatively small number of critical exposures of human skin to establish the extent to which the more comprehensive animal data are applicable to man.

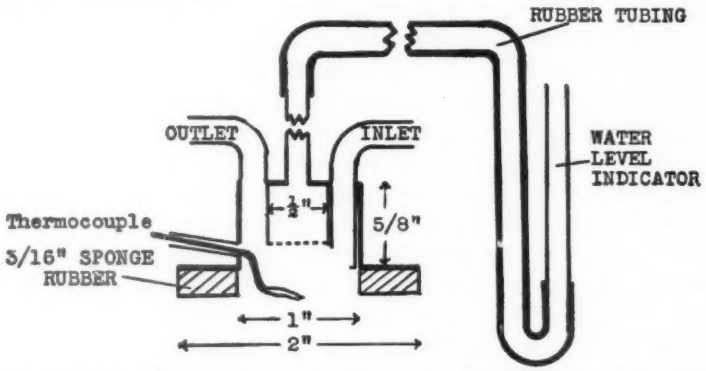
Method of Maintaining Surface of Skin at a Known Degree of Hyperthermia

The method employed by Leach, Peters, and Rossiter [2] for the production of burns in guinea-pigs was investigated and found to be unsuited to the purposes of this study. It was discovered that the temperature of the stream of hot water flowing through the upper and midportions of the metal chamber was significantly and variably higher than that of the surface of the underlying skin. As the water flowed from inlet to outlet there remained a relatively static layer of fluid at the bottom of the chamber. Thus, there were interposed two hindrances to the conduction of heat between the site of measured temperature and the surface of the epidermis, one being the metallic base of the chamber and the other being the layer of quiet fluid above it. Thermocouple measurements of the temperature of the underlying skin disclosed it to be as much as 1° lower than that of the stream of water at the level of the thermometer. In consideration of the fact that the rate at which burning occurs is almost doubled for each degree rise in temperature between 44° and 51°C., the desirability of employing a more precise method of controlling the temperature of the skin is obvious. Another reason for rejecting the method used by Leach, Peters, and Rossiter for the production of burns was that the skin was compressed by the metallic base of the chamber during the period of heat transfer. It was our desire to investigate the effects of hyperthermia independently of any physiological artefact that might be introduced by compressive occlusion of dermal capillaries during the period of exposure.

Direct exposure of the surface of the skin to a rapidly flowing stream of hot liquid was chosen as the method best adapted for the acquisition of these data. With this type of exposure, the surface of the skin could be maintained at the temperature desired without the establishment of an appreciable gradient (<0.1°C.) between it and the source of heat. There was no insulation of the surface by a static

layer of gas, liquid or solid, no heat loss through vaporization of surface moisture, and no diminution of sub-surface heat conduction due to vascular occlusion by the application of pressure on the surface. The method was simple to operate and led to remarkably reproducible cutaneous effects.

The applicator by which a running stream of hot water was brought in direct contact with the skin consisted of a metal cup, the brim of which was covered with a pad of closed-cell sponge rubber to insure a watertight contact. By means of an electric pump, water was circulated from a large constant temperature reservoir through the cup, the open end of which was applied to the skin. The rate of flow was regulated by a screw clamp on the inlet tube and by the height of the outlet tube (Text-Fig. 1).



Text-Figure 1. An apparatus for exposing the skin to a flowing stream of liquid. The surface is brought immediately to, and maintained at, a predetermined and constant temperature without altering surface pressure. The apparatus consists of a brass cup, the base of which is open to permit direct contact between heat source and skin. Water (or oil) was heated by a manually operated steam coil in a large reservoir and pumped through the cup. The pressure within the cup was regulated by adjusting the rate of flow and the level of the outlet.

Tangential flow of a liquid produces no vertical component of force and thus no vertical pressure. Vertical water pressure within the cup could be varied between 70 and 86 cm. of mercury by suitable adjustments of the aperture of the inlet and the height of the outlet tubes. A copper-constantan thermocouple measured the temperature of the water flowing next to the skin. During any period of exposure the temperature of the water flowing over the skin could be controlled to within 0.1°C.

Two methods were used to equilibrate the apparatus before applying it to the skin. In one, the apparatus was applied to a block of linoleum, adjusted to the desired pressure, and transferred to the skin site to be exposed as soon as the temperature equilibrium was reached. In the

other, the applicator was allowed to remain immersed in the hot water reservoir with the pump turned on until thermal equilibrium was established. The cup was then transferred immediately to the skin and adjusted to the desired water pressure.

Provision was made in the construction of this apparatus for studying the relation of the size of the area of exposure to the intensity of the resultant injury. This was accomplished by making the brim of the cup removable so that the area of skin to be exposed could be varied according to the aperture selected for use. Thus, in the same region on the same animal and under identical conditions of time, temperature, and pressure, circular targets having a diameter of either 7 or 25 mm. could be exposed.

Individual burns in the animal experiments were 25 mm. in diameter. This was larger than was desirable for human subjects and the diameter of the aperture of the cup was accordingly reduced to 7 mm. for the human experiments. Before doing so, however, it was established by animal experimentation that the reduction in the size of the exposure area did not make an appreciable difference in the effect on the epidermis.

Water was employed as the source of heat in all of the experiments summarized in Table II. Because the question was raised whether a hypotonic fluid such as water might modify the effects of heat, a series of comparable exposures were made in which oil was substituted for water. There was no appreciable difference between the injury-producing potentiality of rapidly flowing streams of water and of oil on either animal or human skin so long as the temperature and duration of exposure were the same.

Experiments on Pigs

The pig was used in these studies because it was found that no other readily available animal has skin that bears so close an anatomical resemblance to that of man.

Porcine Epidermis

The epidermis over the lateral body area of the pig measures approximately 0.1 mm. in thickness. Like that of man there are irregularities in the contour of both the upper and lower surfaces of the epidermis, those on the upper being due to an intricate system of intercommunicating linear depressions and those on the lower corresponding to the dermal papillae over which the epidermis is moulded (Fig. 1 in Study III *).[3]

* Studies of Thermal Injury, III, will appear in the November issue of The Journal.

Like that of man, the outermost zone or stratum corneum of the pig's epidermis consists of several loosely connected layers of desiccated and intensely basophilic remains of keratinized epithelial cells.

The second or granular layer is thin and consists of several layers of dead or dying squamous cells, the acidophilic cytoplasm of which contains many fine, deeply basophilic keratohyaline granules. Many of these cells have lost their nuclei. Others contain shrunken hyperchromatic nuclei or Feulgen-negative nuclear ghosts.

The third zone is comprised of several layers of aging squamous cells which no longer have any direct cytoplasmic attachment to the dermis. The cytoplasm is dense, deeply acidophilic, and appears desiccated. The cells are so closely packed that neither intercellular bridges nor spaces can be recognized. Many of the nuclei are relatively small and more densely packed with chromatin granules than those of the deeper cells.

The fourth zone consists of cells in transition between the squamous and the basal cell layer. The transitional cells are large and polyhedral and many of them still have an attenuated foot-like cytoplasmic attachment to the dermis. It is in this zone that intercellular bridges of tonofibrils are most readily visualized. The cytoplasm is moderately basophilic. The cell outlines are distinct and the intercellular spaces are clearly defined. The nuclei are larger and rounder than those of the more superficial cells and contain several coarse and many fine granules of chromatin.

The fifth zone is comprised of the basal cells which, save for their cuboidal or columnar shape and their palisade-like arrangement on the dermis, are essentially similar to the overlying transitional cells. Projecting from the inferior surface of the basal epidermal cells of the pig are many robust tonofibrils which appear to be embedded in the dense feltwork of fine collagen fibrils that comprise the superficial zone of dermis. No such fibrillar anchorage of epidermis to dermis can be seen in human skin (Figs 1 to 6 in Study III).[3]

The microscopic appearance of the epidermis of both man and pig suggests that there is a progressive loss of intracellular water as the epithelial cells grow older and move away from the dermis. The nearer the surface the more desiccated the cells appear. The entire stratum corneum and most of the cells of the granular layer appear to be incapable of vital reaction.

Porcine Dermis

The dermis covering the lateral body surface of immature pigs measures between 1.0 and 2.0 mm. in thickness and is generally more compact than that of man. In both pig and man the superficial portion

of the dermis comprising the papillary layer or corium is characteristically a soft, thin, loosely arranged feltwork of delicate collagen fibrils in which there appears to be an abundant amount of interstitial fluid. In man it is readily distinguishable from the thick underlying reticular layer which is comprised of robust and closely interwoven bundles of collagen fibrils. Elastic fibrils are more numerous in human than in porcine skin. On the lateral body surface of the pig the corium tends to be thinner and less well defined than it is in man and in places is only slightly less compact than the reticular zone (Figs. 1 to 6 in Study III).[3] The deeper portion of the reticular connective tissue sends trabecular extensions into the underlying adipose hypodermis.

Blood Vessels of Porcine Skin

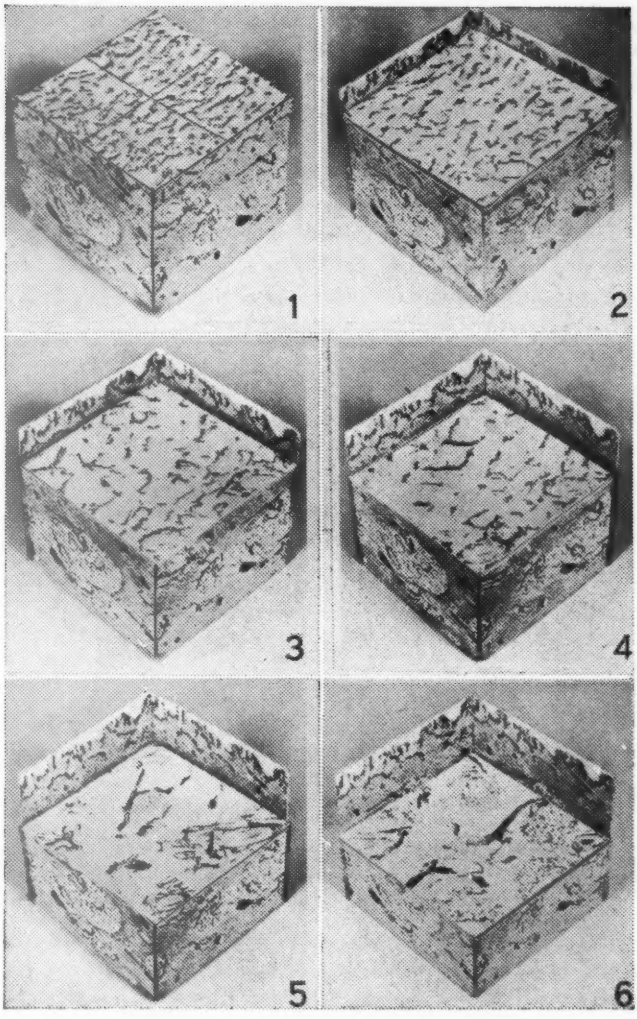
It was observed in ordinary histological preparations that the appearance of the capillaries in the dermal papillae of the body skin of the pig is similar to that in corresponding regions of man. In recognition of the fact that it is difficult or impossible to get an accurate impression of so complicated a structure as a capillary network by two-dimensional visualization, a modification of the Pickworth technic[4] was employed in order that the dermal blood vessels could be studied in three dimensions.

Maximum cutaneous hyperemia of an area of skin was induced by exposing it for 20 minutes to water at 50°C. After such an exposure the erythrocytes were so densely packed in the distended capillaries that there was practically no loss of blood when the skin was incised. Skin and subcutaneous tissue treated in this way was excised to a depth of 8 mm., fixed in 10 per cent formalin, cut in thick sections, and treated with benzidine.

The benzidine imparted a dark blue color to the contents of the engorged vessels. After skin treated in this manner was cleared, a three-dimensional study of its blood vessels could be made with a binocular microscope.

The appearance of the dermal vessels of porcine skin at various levels below the surface is shown in Text-Figure 2. To prepare this illustration a block of benzidine-treated skin was cut serially and parallel to the surface in sections measuring 50 μ in thickness. Another block of the same skin was cut serially and at right angles to the surface. Photographs were made of both series and the prints were mounted in such a manner as to orient the horizontal sections in relation to the depth below the surface that each represented.

In approaching the surface of the body, blood vessels follow an oblique course through the hypodermis and, after reaching the lower



Text-Figure 2. Series of composite photomicrographs showing vascularization of a block of hyperemic porcine skin which measured 2 by 2 by 2 mm. A series of thick (50 μ) benzidine-treated horizontal and vertical sections were mounted in such a way as to show the distribution of veins, arteries, and capillaries at various levels beneath the surface. No. 1 shows the capillary plexus lying in the most superficial (50 μ) portion of the dermis. No 6 shows the vessels in the most superficial layer of the adipose tissue of the hypodermis.

layer of the dermis, branch horizontally to form multiple inter-venal and inter-arterial anastomoses. From these horizontal plexuses there originate a series of broad vascular loops that penetrate to the mid-

portion of the dermis. Inter-arterial and inter-venal anastomoses between these loops serve to establish a mid-dermal plexus. From this mid-dermal plexus originate numerous hairpin-shaped capillary loops which extend upward into the dermal papillae. These capillary loops anastomose freely with one another and constitute the most superficial or papillary plexus. The capillary communications between the superficial arterioles and venules occur at different levels. Some follow a course that brings them within a few micra of the basal epithelial cells over the tips of the papillae. Still others follow an almost horizontal course to establish communications between the arterioles and venules of the intermediate plexus. At all levels through the dermis there are numerous vascular communications with the mantle-like meshwork of capillaries that surrounds the hair follicles and dermal glands.

As may be seen in Text-Figure 2, the number, size, distribution, and communications of the dermal blood vessels of the pig are remarkably similar to those described by Spalteholz [5] in human skin. The similarity of blood vessels in human and porcine skin was found to be so great that it was with difficulty that one could be distinguished from the other in Pickworth preparations.

It is not intended to imply that the anatomical resemblance between the vessels of human and porcine skin denotes an equal degree of functional similarity. Certainly, the vascularization of both indicates that ample and similar mechanical facilities exist either for the transfer of body heat to the surface to facilitate its dissipation, or for the conduct of surface heat to the interior to raise the internal temperature of the body.

Sweat Glands and Sweating

Several types of glands are encountered in the dermis of the pig and although one of them bears some resemblance to the sudoriferous glands of human skin, it does not secrete a significant amount of sweat.

The fact that the pig does not sweat was verified by a series of experiments in which the water loss from the skin of living pigs was measured at various environmental temperatures, with and without the administration of pilocarpine (Table I).

It was found that the water loss from the skin of a live pig does not differ significantly from that of one that is dead. In a cool environment the water loss per square cm. per minute is approximately the same in man and pig. At higher environmental temperatures the rate of water loss from human skin is tremendously augmented, whereas the corresponding increase in water loss from the skin of a pig is relatively small and is due to more rapid evaporation of tissue water rather than to sweating.

So far as can be judged by anatomical criteria, the pig should be a suitable experimental subject from which to derive certain types of information regarding the effects of heat on human skin. Its various layers are of comparable thickness and structure. Its blood vessels are similar in size, number, and distribution. As will be shown later, its susceptibility and reactions to control episodes of hyperthermia are remarkably similar to those of man.

TABLE I

Rate of Water Loss from Surface of Human and Porcine Skin *

	Water loss (mg. per sq. cm. per minute) during a period of 10 minutes							
	Temp., 21°C.; humidity, 30 to 40%				Temp., 36°C.; humidity, 30 to 40%			
	No. of tests	Minimum	Maximum	Mean	No. of tests	Minimum	Maximum	Mean
Dead pig (lateral thoracic region)	4	0.016	0.026	0.019	4	0.023	0.031	0.027
Live pig (lateral thoracic region) without pilocarpine	5	0.016	0.028	0.021	6	0.020	0.032	0.028
Live pig (lateral thigh):								
Without pilocarpine					4	0.018	0.026	0.024
†With pilocarpine (1 mg. per kg. of body weight)					4	0.021	0.030	0.027
Live man (forearm):								
Subject #1 (A.R.) without pilocarpine	1			0.027	1			0.180
Subject #2 (A.M.) without pilocarpine	2	0.028	0.038	0.033	2	0.280	0.360	0.320

* Amount of water loss was determined by accretion in weight of $Mg(Cl\ O_4)_2$ contained in base of weighing bottle during the time that the neck of the bottle was held in contact with the skin.

† Iodine color test negative.

Since a pig does not sweat, allowance should be made for the inability of porcine skin to lose heat through the vaporization of moisture derived from sweating. The significance of heat loss through vaporization of moisture in respect to cutaneous burning will be discussed in greater detail in study IV of this series.[6]

Thermal Exposures of Porcine Skin

Closely clipped young (8 to 10 kg.) white pigs were used. It was found that the skin of the pig was not uniformly susceptible to thermal injury. That covering the ears, thighs, buttocks, and ventral surface was more, and that of the neck and midportion of the back less vulnerable, than was the skin of the lateral portion of the shoulders, thorax, and abdomen. The largest uniformly reacting area was the lateral body surface beginning anterior to the thighs and extending forward over the shoulders.

TABLE II

Time-Surface Temperature Thresholds for Thermal Injury of Porcine Skin

Temperature in °C.	Time		Number of experiments	Sub-threshold exposures				Threshold and supra-threshold exposures	
				1° reactions				2° and 3° reactions	
				Hyperemia only		Focal epidermal necrosis		Complete epidermal necrosis	
	Minutes	Seconds		Mild	Severe	Scaling	Small ulcers	Red burn	Pale burn
44	420		1					x	
45	150		1	x					
	180		1					x	
46	45		1	x					
	60		1		x				
	90		1					x	
46.5	45		1	x					
	60		1					x	
47	35		1	x					
	45		1					x	
	50		1					x	
	60		1					x	
48	10		3	x					
	12		1		x				
	14		2			x			
	14		1					x	
	15		2					x	
	16		1		x				
	18		1					x	
	20		1					x	
52	2		4					x	
Cont.	3		1					x	
53		20	1	x					
		30	1		x				
		45	2			x			
	1		2					x	
	1	30	3					x	
	2		1					x	
54		15	1	x					
		25	1		x				
		35	1					x	
55		5	1	x					
		10	1	x					
		15	1	x					
		20	1			x			
		25	1					x	
		30	3					x	
56		10	1			x			
		15	1					x	
		20	1					x	

49	3		4	x					
	4		5	x					
	5		2	x					
	6		5		x				
	6		2			x			
	6		2				x		
	7		2		x				
	7		1			x			
	7		1				x		
	8		4			x			
	8		1				x		
	8		2					x	
	9		11					x	
	10		5					x	
50	1		1	x					
	2		1	x					
	4		1			x			
	5		1		x				
	5		3			x			
	5		2				x		
	5		2					x	
	6		2					x	
	6	30	2					x	
51		45	2	x					
	1		2	x					
	1	30	2	x					
	2		1			x			
	3		2		x				
	3		2			x			
	3		2					x	
	4		2					x	
	5		1				x		
	5		1					x	
	10		2					x	
52		30	1	x					
		45	1		x				
	1	30	1					x	

58		5	1	x					
		10	1					x	
60		2	1	x					
		2	1			x			
		3	1			x			
		5	1					x	
		7	1				x		
		7	1					x	
		10	2					x	
	10		1					x	
65		1	1	x					
		2	1					x	
		3	1					x	
	10		1					x	
70		1	2					x	
		2	1					x	
	3		2						x
75		1	1					x	
		5	1						x
80		1	1					x	
		5	1						x
85		1	1					x	
		5	1						x
90		1	1					x	
		5	1						x
95		1	1					x	
		3	1						x
100		1	1					x	
		3	1						x

The results of 179 exposures of pigs' skin with temperature and duration of each are shown in Table II. All animals were first anesthetized by intraperitoneal injection of pentobarbital sodium.

The surface temperatures at which these exposures were made ranged between 44° and 100°C. The duration of exposures varied between 1 second and 7 hours. The majority of the exposed sites were kept under observation until the reaction had subsided or the lesion had healed. In the case of borderline reactions, duplicate exposures were made and the areas excised at the end of 24 or 48 hours for microscopical study. As indicated in Table II, a wide variety of reactions was observed. These ranged in severity from evanescent erythema to deep necrosis.

It was found that all exposures fell into one of two groups according to whether they had caused full-thickness destruction of the epidermis over the entire target area. Those that failed to cause complete trans-epidermal necrosis were designated as sub-threshold. Those that resulted in complete trans-epidermal necrosis were designated as threshold or supra-threshold depending on whether they were just sufficient or more than sufficient to destroy the epidermis.

Reactions to exposures that were of insufficient intensity or duration to cause complete destruction of the epidermis were designated as first degree. In the mildest of these, the total response to the episode of hyperthermia was evanescent dilatation of superficial cutaneous blood vessels. In others, the hyperemia was more intense and prolonged. In still others, the occurrence after a few days of excessive exfoliation or focal ulceration indicated that some of the exposed epidermis had sustained irreversible injury.

Cutaneous reactions indicative of full-thickness destruction of epidermis over the entire target area were designated as second or third degree according to the depth to which irreversible injury was estimated to have occurred. If the clinical course or microscopic appearance of a lesion indicated that trans-epidermal necrosis had occurred without a significant amount of irreversible damage to the dermis, the reaction was designated as second degree. The more any given exposure exceeded in either duration or intensity the threshold at which the epidermis was destroyed, the greater the depth to which the dermis was affected. Reactions indicating that a significant degree of irreversible injury to the dermis had occurred were designated as third degree. In all second and in many third degree reactions the burned skin was visibly hyperemic for many days. In some third degree reactions the surface of the burn became immediately ischemic and re-

mained so until the pale and necrotic layer of superficial tissue was detached.

In the beginning there was some difficulty in the establishment of clinical criteria by which to predict the ultimate severity of certain injuries. Although there was no difficulty in recognizing almost immediately the difference between a reaction of which the total effect was a mild and transient erythema and one that consisted of deep coagulation necrosis, it was not always possible during the first few days to recognize by clinical observations whether a given lesion represented a severe first degree reaction with incomplete or focal epidermal destruction or a relatively mild second degree reaction.

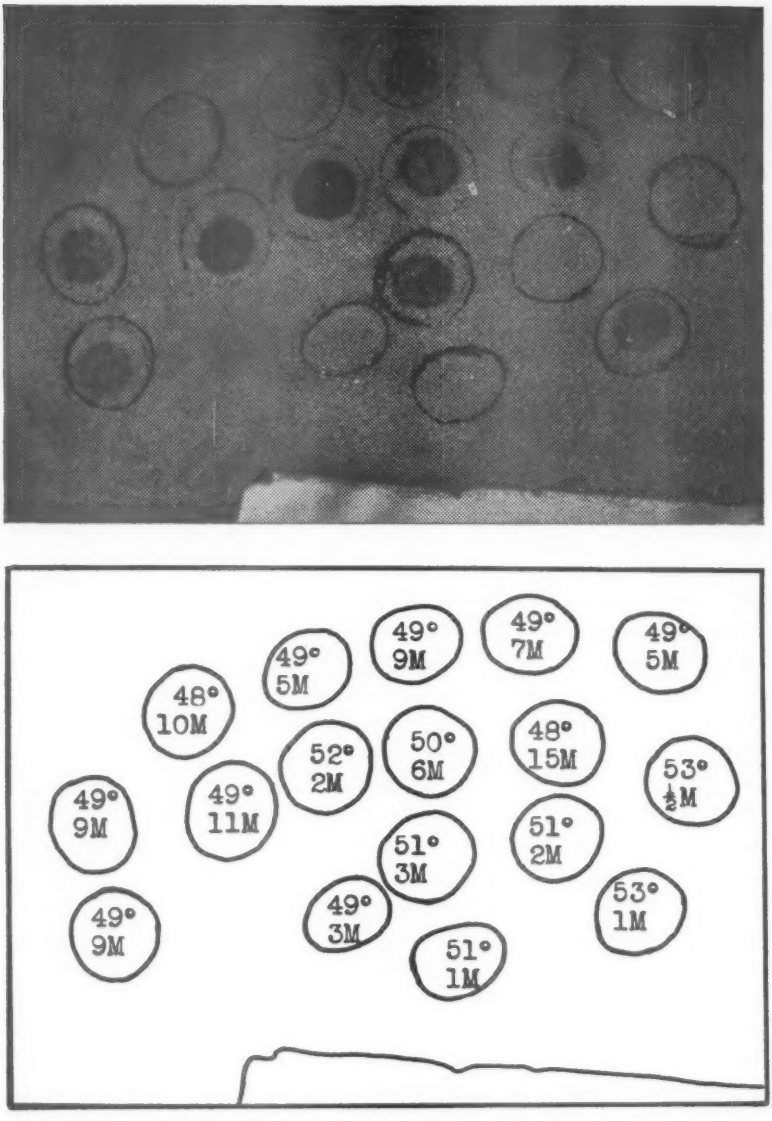
Apart from the microscopic appearance, the most reliable criteria by which to recognize trans-epidermal necrosis were (a) the ease with which dead but still intact epidermis could be displaced by friction on the second and third days after exposure, and (b) the development of complete encrustation of such a lesion within a week.

The macroscopic appearance of different degrees of cutaneous reaction to hyperthermia may be seen in the photographs of the right and left sides of pig 924 in Text-Figure 3, made when the lesions on the right side were 24 hours old and those on the left were 7 days old. It is apparent from these photographs that the duration of exposure at any given temperature was remarkably critical in relation to the kind of reaction evoked. It is equally apparent that the time required to produce a given degree of reaction varied inversely with the temperature.

Experiments on Human Subjects

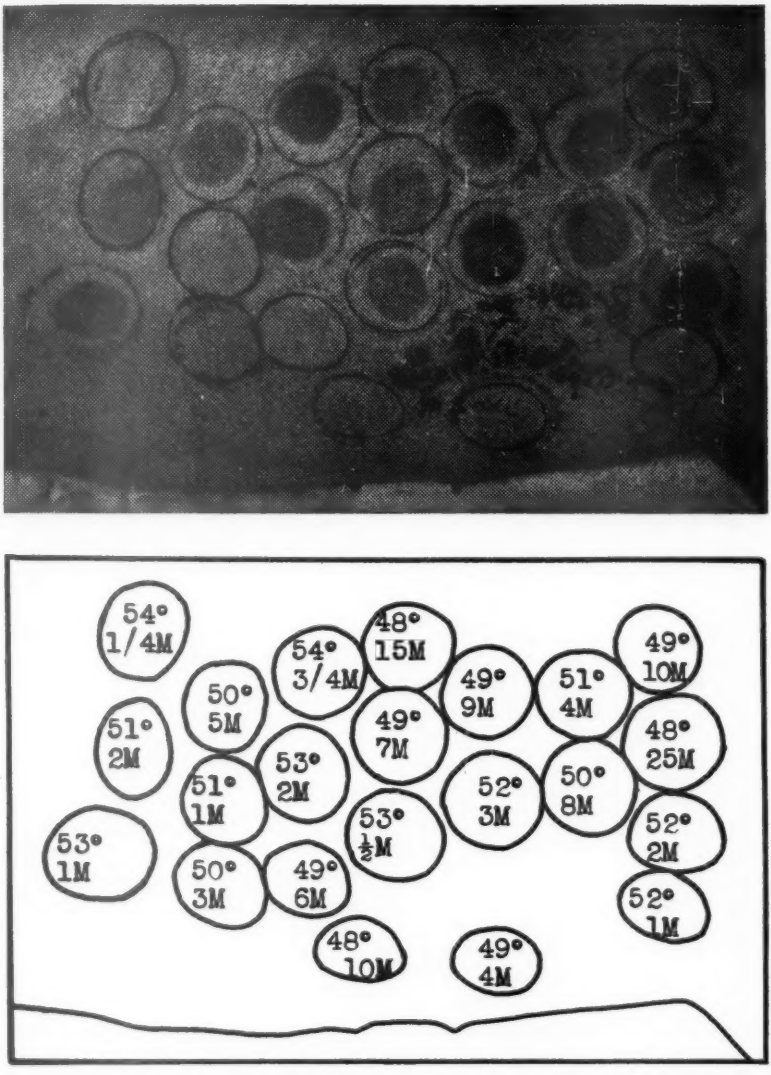
In order to determine the extent to which the results of experiments on pigs were applicable to man, a series of 33 exposures were made on human volunteers (Table III). In some the heat was applied to the skin of the anterior thoracic region and in others to the ventral aspect of the forearm. The exposures were made with the apparatus shown in Text-Figure 1.

As in the pig, the reactions of human skin to hyperthermia were designated as first, second, or third degree. Reactions characterized as first degree were those in which part or all of the epidermis escaped irreversible damage. At one extreme a first degree reaction consisted of nothing more than transient hyperemia. At the other, the erythema was more severe and prolonged and was followed by the formation of miliary vesicles which did not coalesce. Lesions in which there was complete necrosis of the epidermis over the entire target area were designated as second or third degree reactions, depending on the depth



Text-Figure 3-a. Photograph and diagram of burns on the left side of a pig, with the temperature and duration of exposure indicated. Lesions are 7 days old.

to which the dermis appeared to have been destroyed. As in the experiments on pigs, a threshold exposure represented the shortest time at any given temperature that caused complete destruction of the epidermis.



Text-Figure 3-b. Photograph and diagram of burns on the right side of a pig, with the temperature and duration of exposure indicated. Lesions are 24 hours old.

That a given exposure of human skin had resulted in trans-epidermal necrosis was usually, but not always, recognized by early and complete vesication of the target area. Although vesication indicated that the epidermis had been destroyed, absence of vesication did not always indicate epidermal survival. In several instances trans-epidermal necrosis occurred without vesication after supra-threshold exposures.

The explanation of this phenomenon will be discussed subsequently in relation to the pathogenesis of burns.

Discomfort in the form of a stinging sensation occurred between 47.5° and 48.5°C. and was felt more intensely by some subjects than

TABLE III

Time-Surface Temperature Thresholds for Thermal Injury of Human Skin

No.	Temp. at surface in °C.	Duration of exposure			Sub-threshold exposures	Threshold and supra-threshold exposures	Subject	Date
					1° reactions	2° and 3° reactions		
		Hours	Minutes	Seconds	Hyperemia without loss of epidermis	Complete epidermal necrosis		
1	44	5			x		BF	2/6
2*		5			x		BF	2/23
3		6				x	BF	2/6
4*		6				x	BF	2/23
5*	45	2			x		KL	2/16
6*		3				x	KL	2/3
7		3				x	HA	2/4
8*	47		18			x	RK†	2/13
9*			20		x		KL	2/25
10*			20		x		AM	2/26
11*			20		x		PG	2/26
12			25			x	RK†	1/8
13*			40			x	AM	2/26
14			40			x	PG	2/26
15			45			x	RK†	1/8
16	48		15		x		PG	7/19
17			15			x	AR	7/19
18			18			x	AM	6/26
19*	49		8		x		AM	2/16
20			8		x		AM	6/26
21			9	30		x	AM	6/26
22*			10			x	AM	6/26
23			11			x	AM	6/26
24			15			x	AM	6/26
25	51		2		x		AM	6/26
26			4			x	AM	6/26
27			6			x	AM	6/26
28	53			30	x		AM	6/26
29			1	30		x	AM	6/26
30	55			20	x		PG	7/19
31				30		x	AR	7/19
32*	60			3	x		FH	2/1
33*				5		x	FH	2/1

* Oil used instead of water as source of heat.

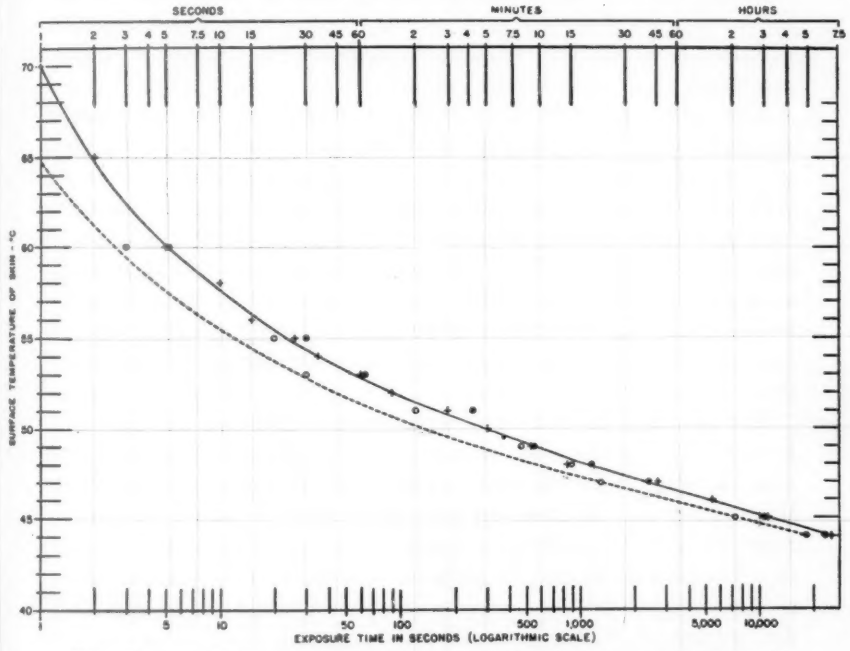
† Subject RK was atypical in that his threshold for thermal injury was significantly lower than that of other experimental subjects.

by others. Severe burns were sustained without discomfort at 47°C. and intense discomfort was sometimes complained of before any irreversible injury had been sustained at temperatures in excess of 48°C.

The results of the human experiments have been summarized in Table III.

Relative Vulnerability of Porcine and Human Skin to Thermal Injury

To facilitate comparison of the data included in Tables II and III, certain of the more critical observations in both have been combined graphically in Text-Figure 4. The solid line was established by points representing the time and temperature of exposures that caused minimal second degree reactions of porcine skin. The points by which this



Text-Figure 4. Time-surface temperature thresholds at which cutaneous burning occurs. The broken line indicates the threshold at which irreversible epidermal injury of porcine skin is first sustained. The solid line indicates the threshold at which epidermal necrosis of porcine skin occurs. Critical exposures of porcine skin are represented by crosses. Each cross denotes the shortest exposure time at the temperature indicated which resulted in trans-epidermal necrosis. The results of critical experimental exposures of human skin are indicated by circles. The open circles represent the longest exposure at the temperature indicated that failed to destroy the epidermis, and the solid circles represent the shortest exposure at the temperature indicated that resulted in trans-epidermal necrosis.

line was established are represented by crosses. Each cross represents the shortest time at the temperature indicated that resulted in trans-epidermal necrosis of the entire target area afer exposure of pig's skin. The more that the time of any given exposure placed it to the right, or that the temperature placed it above the solid line, the greater the

depth to which the skin was destroyed. All exposures that were situated a significant distance above and to the right of the solid line were supra-threshold and all those situated a significant distance below and to the left of the solid line were sub-threshold.

The extent to which the reactions of human exposure corresponded to those observed in the more comprehensive animal experiments is indicated by the open and solid circles in Text-Figure 4. The open circles represent the maximum exposures that failed to destroy human epidermis and the closed circles represent the minimum time at the temperature indicated that resulted in complete destruction of human epidermis.

The broken line in Text-Figure 4 represents the approximate threshold at which the first morphological evidence of thermal damage to porcine epidermis was recognized. Exposures situated below the broken line caused no appreciable injury. Exposures lying between the broken and solid lines resulted in varying degrees of epidermal damage short of trans-epidermal necrosis. Since the reaction of human skin to controlled episodes of hyperthermia was not examined microscopically, no inferences can be drawn as to the precise time at any given temperature at which microscopic evidence of injury to human epidermis was first recognizable.

The results of the two sets of experiments (Tables II and III) indicate that at similar surface temperatures there is little or no quantitative difference in the susceptibility of human and porcine epidermis to thermal injury. The time-surface temperature threshold for the occurrence of trans-epidermal necrosis in man appears to be similar to that for the pig. It may be inferred that the optimal thermal milieu of the epidermal cells of both man and pig lies within a few degrees of the temperature that is normal for their internal tissues and that any rise in epidermal temperature above that level may be injurious if sufficiently prolonged.

The lowest surface temperature that was responsible for cutaneous burning in these experiments was 44°C. and the time required to cause irreversible damage to epidermal cells at this temperature was approximately 6 hours. It could be inferred from the contour of the curve (Text-Fig. 4) which represents the injury-producing threshold that burning would probably have occurred at even lower temperatures if the experiments had been sufficiently prolonged. The rate at which irreversible cellular injury was sustained increased rapidly as the surface temperature was raised, and for each degree rise in surface temperature, between 44° and 51°C., the time required to produce such injury was reduced by approximately one-half.

Above 51°C. the rate of injury began to fall off and the time-temperature curve depicting the threshold at which trans-epidermal necrosis occurred tends to become asymptotic in relation to the temperature axis. Below 44°C. there was a rapid decrease in the rate at which burning occurred and the time-temperature curve depicting the threshold for burning becomes asymptotic in relation to the time axis.

Through reference to Text-Figures 1 and 2 in Study III,[3] it will be apparent that the change in trans-epidermal temperature caused by exposing the surface of the skin to excessive heat is comprised of two phases. The first represents the time required to satisfy the thermal capacity of the epidermis or the transfer of a sufficient amount of heat energy to establish a stabilized trans-epidermal gradient. Thirty seconds was ordinarily sufficient for the attainment of a steady state of heat transfer in which the temperature at the basal cell level was only slightly lower than that at the surface. The second phase represents that part of the hyperthermic episode in which the trans-epidermal temperature gradient was stabilized.

Thus, in the case of surface temperatures under 51°C. the time required to cause irreversible injury of the epidermis was so long in relation to the amount of time required to bring the temperature of the basal cell level to a steady state that the latter was negligible. The total exposure time required to destroy the epidermis at such surface temperatures was essentially identical to the total duration of the steady thermal state within the epidermis, and under these circumstances there was a linear relationship between time and surface temperature in the production of burns between 44° and 51°C.

The reason that this linear relationship did not prevail below 44°C. probably was due to the increased effectiveness of the cellular reparative processes as the hyperthermic level approached the temperature range that was normal for the tissue.

As surface temperature rose above 51°C. and the total periods of exposure were shortened, the 30 seconds required to stabilize the epidermal temperature came to represent a progressively larger proportion of the entire hyperthermic episode. There was no longer the same kind of relationship between the surface temperature and that at the basal cell level as existed with the longer exposures and there was a progressive deviation from the linear relationship of surface temperature and time that characterized the injury curve between 44° and 51°C.

It should be borne in mind that these data refer to surface rather than to environmental temperature and it is not intended to imply that identical circumambient temperatures necessarily result in identical

surface temperatures of human and porcine skin. The only inference that is justified is that at any given surface temperature the time required to destroy porcine epidermis is approximately the same as that required to destroy human epidermis.

A mathematical analysis of these and other data and a consideration of their significance in relation to the rate processes of other physicochemical phenomena are included in Study V of this series.[7]

Vulnerability of Ischemic Skin to Thermal Injury

One of the reasons that exposure of the skin to a running stream of hot water was the method of choice in these experiments was the belief that by this technic there would be no mechanical interference with the circulation of blood through the dermal capillaries. All of the foregoing exposures were made at atmospheric pressure. It was believed that circulation of relatively cool blood through the dermal capillaries probably would tend to protect the skin against burning and that to be applicable to field conditions data on the tolerance of skin to hyperthermia should be derived from the reactions of physiologically normal tissue.

In order to determine the extent to which local impairment in blood flow may increase the vulnerability of the epidermis to thermal injury, the following experiments were undertaken.

A control series of burns was made on each of 3 pigs by exposing various skin sites to running water at atmospheric pressure. The predetermined time and temperature of each exposure was such that severe first degree or mild second degree reactions could be anticipated (Table IV).

It was found that all 7 minute exposures at 49°C. and all 2 minute exposures at 51°C. made at atmospheric pressure were sub-threshold in the sense that they failed to cause complete trans-epidermal necrosis. That they were close to threshold was indicated by the fact that all 9 minute exposures at 49°C. and all 4 minute exposures at 51°C. did cause trans-epidermal necrosis.

Having established the position of the threshold for trans-epidermal necrosis in these animals to be between 7 and 9 minutes at 49°C. and between 2 and 4 minutes at 51°C. for exposures made at atmospheric pressure, a second series of exposures was now made in which the water pressure was increased by an amount corresponding to 80 mm. of mercury. With this pressure on the surface of the skin during the time that it was exposed to heat, there was no instance in which the reaction to a 7 minute exposure at 49°C. or to a 2 minute exposure at 51° C. was increased in severity.

It is apparent from the data summarized in Table IV that the application of pressure sufficient to collapse superficial dermal capillaries during a period of exposure does not cause appreciable augmentation in the vulnerability of epidermis to thermal injury.

In view of the extreme thinness of the epidermis, these results were to be expected. For reasons discussed in Study I of this series,[8] the temperature of the basal cell layer of the epidermis is determined primarily by the temperature of the surface. Thus, the dermal tem-

TABLE IV

Effect of Thermal Exposures with and without Pressure Ischemia

Animal no.	Temperature	Duration of exposure	Excess pressure on skin	No. of exposures made	Number of lesions	
					Without trans-epidermal necrosis	With trans-epidermal necrosis
	(°C.)	(*minutes*)	(*mm. Hg*)			
887	49	7	0	5	5	0
	49	9	0	5	0	5
	49	7	80	5	5	0
899	49	7	0	4	4	0
	49	8	0	4	2	2
	49	9	0	4	0	4
	49	7	80	4	4	0
	49	8	80	4	3	1
901	51	2	0	3	3	0
	51	3	0	3	2	1
	51	4	0	3	0	3
	51	2	80	3	3	0
	51	3	80	3	1	2

perature gradients, which may be appreciably altered in ischemic as compared to normal skin during thermal exposure, would have little effect on the time-temperature relationship that exists at the epidermal-dermal interface.

LATENT THERMAL INJURY AND THE CUMULATIVE EFFECTS OF REPEATED SUB-THRESHOLD EXPOSURES

When the data summarized graphically in Text-Figure 4 are recalled, it is apparent that morphologic cellular alterations occurred only during the terminal phase of sub-threshold exposures. Not until the duration of any given episode of hyperthermia was such as to bring it to the level indicated by the interrupted line in Text-Figure 4 was there recognizable evidence of epidermal injury. This phenomenon is even more readily apparent in the photographs shown in Text-Figure 3. In these it may be seen that the 7 minute exposure at 49°C. on the left side of the animal shows only a trace of residual erythema whereas

TABLE V

The Cumulative Effects of Repeated Sub-Threshold Thermal Exposures on the Skin of the Pig*

Duration of each exposure	No. of exposures at same site	Interval between exposures	Effect of exposure on skin				Reference no.
			No evidence of epidermal injury		Epidermal necrosis		
			Mild vascular reaction	Severe vascular reaction	Focal	Complete and irreversible	
(*minutes*)							
3	1		x				1
3	1		x				2
3	1		x				3
4	1		x				4
5	1		x				5
6	1			x			6
6	1			x			7
6	1			x			8
7	1			x			9
7	1				x		10
8	1				x		11
8	1				x		12
8	1					x	13
9	1					x	14
9	1					x	15
9	1					x	16
9	1					x	17
9	1					x	18
3	3	3 min.				x	19
3	3	3 min.				x	20
3	3	3 min.				x	21
3	3	6 min.				x	22
3	3	12 min.				x	23
3	3	24 min.				x	24
3	3	48 min.				x	25
3	3	48 min.			x		26
3	3	72 min.			x		27
3	3	72 min.			x		28
3	3	96 min.			x		29
3	3	120 min.		x			30
3	3	240 min.	x				31
3	3	24 hrs.	x				32
3	3	48 hrs.	x				33
2	5	2 min.				x	34
2	5	30 min.	x				35
2	5	60 min.	x				36
3	2	12 min.		x			37
5	2	60 min.				x	38
5	2	240 min.			x		39

* All exposures were made to water at 49°C.

both of the sites of 9 minute exposures at that temperature show trans-epidermal necrosis. Does this indicate that no epidermal injury had been sustained during the first 7 minutes, or does it mean that injury was present but unrecognizable?

In order to gain more information concerning this point, the experiments summarized in Table V were undertaken. Thermal expo-

sures were made with a running stream of hot water at 49°C. and at atmospheric pressure. Three young pigs were used.

The first series of exposures (reference nos. 1 to 18) were for control purposes and served to establish the reproducibility of reactions to single exposures at this temperature. It may be seen that there was not a single instance in which an exposure for less than 7 minutes caused recognizable necrosis of the epidermis, and that in every instance in which exposures as long as 9 minutes were given there was complete necrosis of the epidermis. Skin sites receiving 7 minute exposures recovered with incomplete or no damage to the epidermis, whereas skin sites receiving 9 minute exposures underwent complete ulceration.

The control exposures were followed by a series (nos. 19 to 39) in which repeated exposures, individually incapable of causing recognizable epidermal injury, were applied to the same area. It was found, for instance, that although a single 3 minute exposure at 49°C. caused no recognizable change in the epithelial cells, three such exposures separated by recovery periods as long as 24 minutes had the same total destructive capacity as a single continuous 9 minute exposure.

It was clear that a certain amount of epidermal injury was sustained during the first 3 minutes and that at least 24 minutes were required before there was an appreciable recovery from this injury. That complete recovery occurred after a period of 2 to 4 hours was indicated by experiments 30 and 31.

Experiments 34 to 39 showed what might have been expected; namely, that recovery from the latent injury of a 2 minute exposure was more rapid and that from a 5 minute exposure less rapid than was the case after a 3 minute exposure.

Further discussion of the implications of these experimental results will be found in Study V of this series.[7]

Summary

The reciprocal relationships of surface temperature and duration of hyperthermia in the production of cutaneous injury have been investigated for pig and man. The data were derived from experiments in which the surface of the skin was brought immediately to, and maintained at, a constant hyperthermic level in such a manner that there was no external mechanical interference with the flow of blood through the skin.

Although there were certain qualitative differences in the reactions of human and porcine skin to excessive heat, there were no significant quantitative differences in their susceptibility to thermal injury in these circumstances.

Time and Temperature in Relation to the Occurrence of Cutaneous Burning

In order to characterize any episode of hyperthermia as critical in respect to its capacity to destroy the epidermis, it is necessary to know both the intensity and the duration of the exposure. When the temperature of the skin is maintained at 44°C., the rate of injurious change exceeds that of recovery by so narrow a margin that an exposure of approximately 6 hours is required before irreversible damage is sustained at the basal cell level. At surface temperatures of 70°C. and higher, the rate of injury so far exceeds that of recovery that less than 1 second is required to cause trans-epidermal necrosis.

At surface temperatures between 44° and 51°C., the total exposure time required to destroy the epidermis is essentially identical to the total duration of the steady thermal state within the epidermis, and, under these circumstances, the rate at which burning occurs is almost doubled with each degree rise in temperature.

Below 44°C. there is a rapid decrease in the rate at which burning occurs and the time-temperature curve is asymptotic in the direction of the time axis. This is probably due to the increased effectiveness of the cellular reparative processes as the hyperthermic level approaches the temperature range that is normal for the tissue.

At surface temperatures greater than 51°C., the exposure time required to destroy the epidermis is so short that during most or all of it the deeper layers of cells are in the process of being brought to, rather than being maintained at, a state of thermal equilibrium with the surface. Thus, as the surface temperature is raised above 51°C., the rate of injury begins to fall off and a time-temperature curve depicting the threshold at which trans-epidermal necrosis occurs is asymptotic in the direction of the temperature axis.

The minimum time required to destroy the epidermis at surface temperatures above 70°C. was not determined. It was observed, however, with exposures at flame temperatures (over 1000°C.), that the amount of time required to raise the temperature at the epidermal-dermal junction to a cell-killing level is so brief that the interposition of anything capable of impeding heat transfer to the skin may be sufficient to make the difference between burning and absence thereof.

Compressive Hyperthermia

Although pressure may increase the rate of heat transfer to the skin, and thereby the rate of burning, by improving the interface contact between it and a solid hot object, there was no evidence that compressive

occlusion of dermal blood vessels has any effect on the susceptibility of the epidermis to thermal injury. When hot water was applied to the surface of the skin at different pressures, it was observed that compressive ischemia did not alter the rate at which burning occurred. It was concluded that the conduction of heat energy away from the skin surface by way of the blood stream does not afford a significant degree of protection against epidermal injury.

Color of Cutaneous Burns

Compression of the skin during exposure to heat may alter the surface color of the resulting burn without affecting its severity. Within a certain range of surface temperature, the application during the exposure of sufficient pressure to blanch the skin may cause a burn to remain ischemic that would otherwise be hyperemic. In such circumstances, differences in color are not indicative of differences in the depth of the injury.

In burns produced without concomitant compression of the skin, the color of the surface of the burn is determined in part by the rapidity and degree of the initial increase in dermal temperature and in part by the duration of the exposure. The surface color of such burns is not a useful criterion for estimating either the severity of injury or the amount of blood that may be pooled in the underlying tissue. When the temperature of the dermis is raised slowly, the superficial vessels become engorged and retain their blood even though the tissue is subsequently coagulated by progressive increase in the intensity of the hyperthermia. When the initial rise in dermal temperature is rapid and high, the superficial vessels contract so quickly that there is no opportunity for them to become hyperemic. Although such burns are superficially ischemic, there is intense hyperemia of the more deeply situated vessels.

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GRANULAR CELL MYOBLASTOMA *

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There are several groups of "debatable tumors" whose origin and behavior in the human body are not yet clearly understood. The rarity of some of them has been responsible for lack of accurate definition and separation. Additional information concerning their structure or clinical course is not, therefore, superfluous. The "granular cell myoblastoma" is a tumor of this type. In his original study in 1926 Abrikossoff [1] considered such swellings as degenerative lesions following injury or inflammation. Since then, evidence has been forthcoming which suggests their neoplastic nature and their origin from tissue having competence to develop into striated muscle. In 1931, as a result of a further study, Abrikossoff [2] revised his opinion and suggested that these tumors had their origin in embryonic muscle cells. Gray and Gruenfeld [6] have objected to this interpretation and have stated that "the term myoblastoma should be discarded for this tumor group," as the evidence on which such histogenesis is based is insufficient and the presumed resemblance to embryonal skeletal muscle illusory. These tumors, with rare exceptions, are believed to be stationary or at any rate very slow-growing. They show a predilection for growth in the oropharyngeal region. It has been suggested that they cannot be completely separated from other muscle tumors and that transitions between rhabdomyoblastomas and these tumors are sometimes seen. In a recent study [4] data regarding such tumors from 162 persons have been tabulated and the opinion has been expressed that "definite conclusions as to the histogenesis of the myoblastomas are not warranted at the present time."

During the last 5 years, 10 such tumors were seen at the Tata Memorial Hospital. Only 6 are dealt with here: 2 were encountered in an unusual location, 3 provide interesting information concerning a malignant course in this type of tumor, and one illustrates an interesting histological structure. The remaining 4 resemble closely similar tumors reported in the literature.

REPORT OF CASES

CASE 1

A married Sinhalese woman, 32 years old, was referred to the Tata Memorial Hospital (no. 3513) from Colombo, Ceylon, for radiation therapy in December, 1942. She gave a history of having fallen down a flight of five steps in March,

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1939, landing on her right hip. She suffered from occasional rheumatic pains in the hip, which were relieved by rubbing. In December, 1940, she noticed a small lump (2.5 cm. in diameter) in the right buttock. This gradually increased. The pain in the thigh became more noticeable and ran down the leg into the right calf, but was never severe. She lost about 10 lbs. in weight. In September, 1942, the tumor was ovoid, about 7 by 6 cm. in surface extent, painless, attached to the gluteal muscles, and not fixed to the bone or skin. Roentgenograms did not reveal alteration in the bony structures. A clinical examination failed to show a tumor in the pelvis or elsewhere in the body. The tumor was removed (Dr. Pieris) under general anesthesia. It was embedded in the substance of the gluteus medius muscle and its tendon. It had "the consistency of liver" and was well encapsulated. The surface was not nodular. The wound healed satisfactorily and the patient improved in general health. The tumor was diagnosed as a "malignant endothelioma."

TABLE I

Data Concerning 10 Tumors of Myoblastomatous Nature

Case no.	Location	Age	Sex	Duration
1	Right buttock	32	F	2 years
2	Right buttock	25	M	3 months
3	Left thigh	26	M	3½ months
4	Occipital bone	32	M	6 months
5	Temporal bone	20	M	10 months
6	Middle ear	72	F	1 year
7	Dorsum of tongue	30	F	2 years
8	Left breast	24	F	4 months
9	Breast	31	F	3 years
10	Right loin	42	M	1½ years

A crescentic scar, 25 cm. long, was seen on the outer aspect of the right buttock on examination in December, 1942. The scar was convex downward with its apex about 3 cm. above the great trochanter. There was a moderate loss of substance beneath the scar without evident muscular or sensory disturbance. A little thickening and adherence in the lowest portion of the scar was noticed. At the same time a spherical, hard, movable nodule (2.5 cm. in diameter), which moved with deglutition, was felt in the substance of the thyroid gland. Laboratory investigations were negative. The basal metabolic rate was —11.5 (Mayo normal standard), +1.5 (Indian standard). A piece of the neoplastic tissue was sent to us for histological study and showed the characteristics which will be described later. The right gluteal region was subjected to a total radiation of 1800 r.* through a single portal, 15 by 12 cm., during the course of 1 week. In August, 1943, a hard mass in the right lateral and posterior wall of the pelvic basin was felt during a follow-up examination. The mass was not tender, the general condition of the patient was good, and she had gained 6 lbs. in weight. Further irradiation of 2000 r. was given in 11 days through a 15 by 15 cm. portal on the posterior surface of the buttock. The mass showed no evidence of regression. One month later a small nodule (1.5 cm. in diameter) was felt in the axilla under the lower border of the right pectoralis major muscle. Both breasts were normal. The nodule was excised a fortnight later and showed the same structure as the original gluteal tumor. At that time the lungs were clear and the nodule in the thyroid gland showed no change. The patient returned to her native place in October, 1943, and succumbed a few months later. There was no autopsy.

* Radiation treatment in all cases was administered with a 200 kv. apparatus, under standard conditions of filters and target skin distance.

Gross Examination

The tumor removed at operation was ovoid, distinguishable from the muscle tissue, and well encapsulated. It was firm and on cutting presented a grayish yellow surface broken up into large and small patches by reddish hyaline strands.

Microscopical Examination

The tumor (Figs. 1 to 3) consisted of sheets of closely packed large cells separated by broad bands of pale edematous collagen. The sheets were composed of clusters of cells grouped in a pseudo-alveolar or organoid pattern. The cells in the group lay in close apposition to each other. The clusters were surrounded by a network of slender fibers interspersed with cells having thin, flattened, elongated nuclei. The tumor cells were large (40 to 60 μ), rounded or polyhedral, and contained coarsely granular cytoplasm which was definitely acidophilic. The granules gave the impression of being packed in parallel rows. The nuclei were vesicular, round or ovoid, with a distinct nuclear membrane and scanty, fine, reticulated chromatin. A small round nucleolus was visible in most cells. A few cells showed two or three nuclei. The nuclei were usually centrally placed. No pigment was seen either in the cytoplasm or in the connective tissue cells. Silver impregnation (Gomori) revealed a delicate reticular stroma surrounding individual cells or small groups. The granules did not stain with sudan IV or scharlach R. The tissue was traversed by a rich network of thin-walled blood capillaries; and plugs of tumor cells appeared to lie in venous channels. Unmistakable longitudinal or cross striations could not be seen in the cytoplasm. Mitotic figures were extremely rare.

CASE 2

A young Hindu Surti domestic servant, 25 years old, was referred to the hospital (no. 3714) because of pain and swelling in the right buttock. The pain had started about 3 months previously and extended down the back of the thigh. He had been treated for sciatica by local doctors without relief. He then noticed a swelling in the buttock which gradually increased in size, leading to a limp and later inability to stand or walk. Clinical examination revealed a rounded, firm, elastic mass (27.5 cm. in diameter) extending from the right iliac crest to the corresponding natal fold. Softer areas could be felt in the substance of the tumor. The tumor could be felt deep in the gluteal muscles and was slightly tender. It was not adherent to the skin and did not fluctuate on palpation. A mass was felt also in the right iliac fossa, close to the bone. All thigh movements were restricted and there was a foot drop on the same side. The right hip joint was not affected and the limbs were equal in measurement.

Blood cells were within normal limits. Chemical examination showed blood sugar, 95.2 mg.; phosphorus, 4.4 mg.; calcium, 10.9 mg.; alkaline phosphatase, 3.1 Bodansky units. There was no Bence-Jones protein in the urine. The Kahn test on the blood was negative. Skiagraphic studies showed an invasion and erosion of the pel-

vic bones at the iliopectineal line on the right side. There were no metastases in other bones or in the lungs. A specimen was excised from the tumor in the buttock. It was firm and elastic and had a uniform gray appearance. The tumor was incorrectly diagnosed as lipogenic sarcoma of bone.

The patient was given X-ray irradiation totaling 2000 r. through a 15 cm. circular portal over a period of 3 weeks. The pain was relieved, the patient became afebrile, but the tumor continued to grow in the right iliac fossa. As the condition of the patient deteriorated steadily, he decided to go back to his native place. Owing to difficulties of transportation during war time, he failed to report again.

Microscopical Examination

The section consisted of bundles of tumor cells separated by fibrovascular connective tissue septa. The cells were large and showed forms varying from globular and ovoid, to ribbon-like, long cylindrical cells. The cytoplasm was acidophilic and showed fine and coarse granularity. There was a definite suggestion of longitudinal and transverse striations in some rare cells, particularly near the ends of long cells in the outer cytoplasmic zones. The nuclei were ovoid, more deeply staining and smaller than in case 1, and tended to lie eccentrically in the cell.

CASE 3

A young Englishman, 26 years old (no. 10164), was admitted for X-ray treatment of a swelling in the left groin. He had noticed a lump 3½ months previously. It was not painful but continued to grow. He was treated at another hospital with sulfonamides and, later, with penicillin. The lump gradually increased and the patient became febrile. An enlarged lymph node was removed from the groin and was reported as "Hodgkin's disease" and another piece removed later as "lymphosarcoma, reticulum cell type." When he was seen at the Tata Memorial Hospital in May, 1945, he had a large, globular, soft mass, 15 by 7 by 5 cm., in the left inguinal region. The skin was adherent to the mass in the region of the scars of previous excisions. Another smaller mass was felt in the left iliac fossa. The patient was much emaciated and was maintaining a temperature of about 100°F. He was given about 1300 r. through a 15 cm. portal over the inguinal and iliac regions in a period of 6 days. His condition grew steadily worse; he became drowsy and unconscious. As radiation was having no effect on the tumor mass, the patient was removed to another hospital where he expired 2 days later.

At autopsy, it was found that, besides the large masses in the inguinal and iliac regions, the peritoneum was studded with numerous firm, grayish white nodules. Lymph nodes of the chain from the left iliac region to the para-aortic region were enlarged. The liver and pancreas showed many tumor nodules. There were numerous secondary deposits in both lungs, particularly along the posterior borders. Except for the heart, other viscera, including the cranial contents, were free from neoplastic tissue. The skin and the mucous surfaces were free from tumor, either pigmented or nonpigmented. The eyeballs were not examined. The heart was slightly enlarged and showed numerous grayish white, firm nodules scattered over the surfaces of auricles and ventricles. They were raised very slightly above the general surface. The size of the nodules ranged from 2 to 7 mm. in diameter. In some places

four or five of these had fused together in a larger mass. On opening the heart (Fig. 4) it was seen that the myocardium was sprinkled with tumor nodules, and many were beneath the endocardium. These nodules seemed more numerous on the papillary muscles, giving them a peculiar beaded appearance. Some of the nodules in the liver were much larger and showed central areas of degeneration. The deposits in the lung and pancreas were similar to those described in the heart.

Microscopical Examination

The mass in the inguinal region and iliac fossa as well as the metastatic deposits in the viscera showed a structure similar to that described in cases 1 and 2. In some areas a pseudo-alveolar grouping was noticeable, and in other fields transitions between the globular and ribbonlike cells were evident (Fig. 5). In some of the lung deposits there was a suggestion of striations in the cells.

Comment

Cases 1, 2, and 3 show many similarities in their clinical behavior and histological structure. They have many features in common and bear resemblance to the published descriptions of "granular cell myoblastoma." All of them were malignant in behavior and fatal in their outcome. They showed a pronounced tendency to spread by the lymphatic pathway. The size of the cells, their polymorphism, the presence of intermediate forms between globular and long cylindrical shapes, the character and situation of their nuclei, and the rich and delicate reticulin network around individual cells suggest their affiliation to the striped muscle cells in the body. However, it must be confessed that in the absence of characteristic striations their definite assignment to the group of muscle tumors may not be possible. Doubt may also be entertained that the tumor in case 3 was not a nonpigmented melanoma, but this seems improbable in the light of other findings in this case. These tumors were all insensitive to radiation and one of them continued to grow during treatment.

CASE 4

A Hindu soldier, 32 years old (no. 8494), was admitted because of a swelling in the skull 5 cm. behind the left ear. He had noticed a small lump in that region since childhood. It was quite painless and caused him no inconvenience until about 7 months previous to admission. At that time the swelling had begun to increase rapidly and had become painful and tender. On examination a hard, nodular, irregular, fixed swelling, 12 by 5 cm., was felt in the left parieto-occipital region. The edges of the tumor seemed to merge imperceptibly with the skull. The scalp was freely movable over the tumor, but the growth of hair over it was scanty. The tumor was tender. There were two small nodes slightly to the left of the midline and about 3 cm. below the occipital protuberance.

Laboratory examination showed no abnormality of the cells of the blood, or in its

sugar, phosphorus, calcium and phosphatase content. The Kahn test was negative. Roentgenograms of the skull (Fig. 6) revealed an area of destruction at the parieto-occipital junction, involving the major part of the left half of the suture line and a small portion of the right side. Both tables of the adjoining bones, especially the occipital, were irregularly destroyed. Sclerosis of the upper part of the occipital bone was evident. The rest of the calvarium was normal. No evidence of increased intracranial tension was found. The findings suggested an involvement of the bones by a neoplasm.

Microscopical Examination

The tumor was composed of sheets or groups of cells separated by bands of rich vascular connective tissue. The tumor cells showed marked polymorphism and variation in size. The cells tended to be arranged in parallel ribbons or compact groups. Most of the cells were large and had pale vesicular nuclei. The nucleoli were not prominent. The cell cytoplasm showed fine and coarse granules and was strongly acidophilic. In some places groups of tumor cells were widely separated and tended to be located around blood capillaries. There was an attempt at an organoid pattern in a few areas (Figs. 7 and 8). Remnants of bone spicules were seen embedded in the neoplastic tissue.

CASE 5

A Parsee college student, 20 years old (no. 10470), was admitted for pain and swelling above the left ear. He had noticed slight pain in the region about 10 months previously and a few days later, a tender swelling. He had consulted various practitioners of medicine and finally an otolaryngologist, who treated him by local applications and internal medication. There had been a steady diminution in the acuity of hearing. On examination, a swelling about 5 cm. in diameter was found above and behind the left ear. This had pushed the pinna downward about 1.5 cm. The contour of the skull bulged about 3 cm. at its most prominent point. The swelling was hard, fixed to the skull, and seemed to be arising from the temporal bone. The scalp was freely movable over it. Clinical and laboratory studies showed no other abnormality. There was no evidence of any other tumor. The skiagrams of the skull (Fig. 9) showed extensive destruction of the squama temporalis and posterior portion of the mastoid process, as well as of adjacent portions of the parietal and occipital bones. The petrous portion was not affected.

Microscopical Examination

A portion of the tumor excised for biopsy showed (Fig. 10) groups and rows of polygonal, cylindrical, and fusiform cells in a richly vascular fibrous connective tissue. The cytoplasm of the neoplastic cells was acidophilic and granular. The nuclei were globular or ovoid and showed variation in size, shape, and staining intensity. There was a suggestion of longitudinal striation in some of the cylindrical cells. The diagnosis was granular cell myoblastoma.

The patient was treated with deep X-rays, with a total dose of 3000 r. through a temporal field, 8 by 10 cm., over a period of 3 weeks. There was a slight but definite regression in the size of the swelling. The general condition of the patient

was good. A second skiagram 1 month later showed an upward extension of rarefaction in the squamous bone. Another course of radiation therapy was administered (total, 3000 r. in 3 weeks). At the end of the treatment pain and swelling were very much reduced. At a follow-up examination 2 months later the patient complained of continuous pain in the left external auditory canal. A skiagram showed spread of the disease into the petrous portion of the temporal bone with increased destruction of bony tissue, but with foci of early sclerosis in the squamous portion of the temporal bone. He was readmitted for treatment 3 months later with unbearable deep pain in the ear. The skiagram showed extensive spread of the disease in the parietal bone.

Comment

In the available literature I have not encountered reports of tumors in the skull such as were found in cases 4 and 5. They have been reported as occurring in facial bones, either the maxilla or the mandible, and mostly in infants at birth. A majority of them were recorded as congenital epulides of the newborn.[4] Tumors of the external auditory canal will be referred to later. The 2 cases reported here afforded interesting material for speculation regarding their histogenesis.

CASE 6

A Parsee woman, 72 years old (no. 14231), was seen with a complaint of pain in the left ear. She gave a history of intermittent purulent discharge from both ears since early childhood. The present difficulty had started about 1 year previously with complete deafness in the left ear. She noticed a little plug of tissue in her outer ear which bled occasionally. Her condition was diagnosed as "polyps" and she was treated with ear drops and repeated removal of projecting bits of tissue, once or twice a week for about 9 months. During this period her pain became worse, and, because it persisted, she saw an otologist who removed the polypoid tissue in the ear and had the pieces examined histologically. They were reported as "fibrous clot in a stage of partial organization." The specialist observed that "the growth was coming from the middle ear, probably the medial wall, but the exact site was impossible to determine; bleeding was not excessive." The patient felt much better after the operation and was relieved of pain and bleeding. A fresh lump began to protrude from the outer meatus within a fortnight. On examination at this hospital it was found that the external auditory meatus was completely filled with a soft grayish white tissue. At the time of examination here she complained of fever, of an obstruction in the left nostril, and of a slight pain on swallowing on that side. A smooth, soft, glandular, polypoid swelling could be seen in the region of the pharyngeal opening of the eustachian tube. It bled easily when touched with a gloved finger. A specimen taken for biopsy from the tumor mass in the external auditory meatus showed the characteristics described below. At operation, as much of the tumor as could be seen was removed from the external auditory meatus. The patient felt much better and her temperature decreased. The tumor mass in the nasopharynx was not altered; the patient is still under observation.

Microscopical Examination

The tumor was composed in its deeper parts of sheets of closely packed, large, pale cells with abundant granular acidophilic cytoplasm. The cells were spheroidal or elongated. The nuclei were relatively small and centrally placed in most cells. There were, however, several

cells showing two or three nuclei huddled together. The cells were held in a delicate reticular and fibrous network with slender capillaries. Mitotic figures were frequent. Some cells showed a suggestion of longitudinal striation, but definite cross or longitudinal striation was not found. Lipoid material could not be demonstrated in the cytoplasm with scharlach R. In the superficial areas there was secondary inflammation with surface necrosis and richly vascular granulation tissue interspersed with isolated tumor cells (Fig. 12).

Comment

Granular cell myoblastomas in the region of the outer ear have been described by Horn and Stout [8] and by Altmann [3] as smooth-lobed or pedunculated, filling the external auditory canal and growing slowly. They attracted attention by bleeding from the ear. A gradual extension involved neighboring structures later in the course of the disease. The case reported here showed extension outward from the tympanic cavity and inward through the eustachian tube. The cells comprising the tumor were more isolated, rounder, and smaller than the benign type of granular cell myoblastoma. In many areas their characteristics were suggestive of a malignant neoplasm and a diagnosis of polymorphous cell sarcoma could not be ruled out definitely.

Discussion

A perusal of the literature on myoblastomas and a study of the pathological material available here have raised questions which may be worth considering.

1. The nature of these tumors, whether they are degenerative processes, productive inflammatory lesions, or true neoplasms, has engaged the attention of many observers. It has even been suggested that tumors of this type are histogenetically dissimilar in different locations and that the myoblastomas of the tongue are unlike those occurring in other regions. These varying opinions lose much of their subtlety when it is realized that in many tumors, "however closely the processes are analyzed, the conclusion remains that inflammatory hyperplasia passes into neoplasia." [5] The close resemblance between the cellular constituents of these tumors and the forms encountered during regeneration of voluntary muscle does not necessarily imply an absence of neoplastic growth but may indicate the various phases during the course of a differentiation of tumor cells. The regenerating tissue following injury or inflammation is not as susceptible as healthy tissue to stimuli which are responsible for maintaining the normal pattern of the organism. It

should therefore be expected that processes leading to repeated destruction and regeneration would also supply the conditions necessary for neoplastic growth. During the past 100 years, pathologists have been obsessed with the idea that neoplasia is an entirely unnatural and abnormal process and not an outcome of interdependent biological events. "When tumor formation is conceived as a necessary, innate and therefore physiological reaction induced by one or other of unnumbered, interchangeable stimuli, and when it is interpreted as resumed if 'pathological' *development*, the mystery commonly said to obscure it is seen to be 'entirely owing to ourselves'." [11] The features which usually characterize a neoplastic growth are that it is progressive, expansive, and infiltrative. In the examples which have been reported above, all of these characters were discernible and there could be no uncertainty regarding their neoplastic nature.

2. The second question concerns their designation. The gross and microscopical characteristics assigned to the group of tumors termed "granular cell myoblastoma" are as follows: They are usually small, circumscribed, encapsulated, spherical, and lobulated masses. Their consistency is firm. On section they present a grayish yellow or tan surface. In some tumors the borders are indistinct and the neoplastic tissue appears to penetrate irregularly in the adjoining muscle. Histological examination shows the tumor to be composed of rather large polyhedral cells, with abundant acidophilic cytoplasm separated in clusters, bundles, or sheets by wide strands of edematous connective tissue. The large size of the cells (50 to 60 μ in diameter) and the coarse or fine refractive granules in the cytoplasm constitute arresting features. The reticulum forms a delicate network between and around individual cells or small groups of cells. The cytoplasm appears red with Masson's trichrome stain and brownish yellow with van Gieson's stain. The granules do not possess the tinctorial properties of glycogen or lipoid. The nuclei are usually small and vesicular, with distinct nuclear membrane and an inconspicuous karyosome. These tumors possess other microscopical characteristics which are interesting. The tumor cells exhibit marked polymorphism with globular cells at one extreme and ribbon-like, long cylindrical cells at the other. Intermediate forms described as teardrop, tadpole, and banjo shapes as well as irregular syncytial masses are encountered. In some tumors there is a marked tendency toward a grouping of cells into pseudo-acinar clusters. These clusters, however, do not show the sharply defined lumina which characterize glandular acini. The presence of longitudinal and cross striations in the cytoplasm of these tumors has given rise to much

discussion. Microscopical appearances highly suggestive of such striations sometimes are met with in the peripheral zones of cells in suitably fixed material.

All of the tumors referred to in Table I satisfy most of the microscopical criteria mentioned above. The histological characteristics as well as the gross appearance of the tumor as it is seen embedded in the substance of a muscle are highly suggestive but not conclusive evidences of its origin from muscle cells. Tissue culture studies have not established the myoblastic nature of these cells. In a personal communication,[13] Dr. A. P. Stout has stated: "Dr. Margaret Murray has grown some of these tumors *in vitro*. They do not behave altogether like striated muscle cells, either benign or malignant. The granular cells wander out and then lose their granules, nor do they reappear again when the cells reproduce." The main difficulty remains as to their relationship, if any, to the rhabdomyomas and rhabdomyosarcomas. A separation of granular cell myoblastomas from myosarcomas appears justifiable in view of a difference in their clinical behavior, which will be referred to later. Abrikossoff [2] (1931) separated a variety with polymorphous cells and areas of frankly sarcomatous nature as his fourth group of myoblastic myomas. Stout [8] has rightly pointed out that this fourth group is really rhabdomyosarcoma and not at all like the first three, because in it the cells are not granular, but resemble those of polymorphous cell sarcomas. He is of the opinion that Abrikossoff had no justification for including the fourth group with the first three.

3. Granular cell myoblastomas have been described as slow-growing benign tumors which do not usually recur after adequate resection. Other observers have found that the incidence of malignancy of "this ordinarily benign tumor cannot at best exceed 10 per cent and is probably not as high as this." [13] Ravich, Stout, and Ravich,[12] while describing their only case of malignant granular cell myoblastoma, emphasized the important histological and clinical distinctions between the benign tumors described by Abrikossoff [2] and the fourth malignant group. They stated that "until the case here reported was observed by us we were firmly convinced that no example of a malignant primary granular cell myoblastoma belonging to any of Abrikossoff's first three groups had ever been recorded." They were satisfied that neither the 5 cases of malignant myoblastoma described by Howe and Warren [9] nor the other 10 culled by them from the literature belonged to Abrikossoff's first three groups in their primary manifestations. In view of this opinion I have pondered over the cases described in this paper and have hesitated before presenting them for fear of recording doubtful

instances of metastasizing granular cell myoblastomas. Repeated study of these cases, however, leaves no other option than to classify them as examples of the third group of tumors described by Abrikossoff. The tumors in the first two patients presented a relatively benign appearance in histological preparations. The cells were large and polygonal, and contained faintly oxyphilic granules which did not show the staining characteristics of lipoid. Case 6 shows many interesting features. Clinically, the tumor was diagnosed for many months as a slow-growing benign papilloma. Microscopic examination showed features associated with active growth and consisted of cells ranging from the large polygonal type, with well marked acidophilic granular cytoplasm, to polymorphous cells showing marked anaplasia, deep-staining nuclei, faintly basophilic homogeneous cytoplasm, and several mitotic figures. The histological features are not so characteristic in cases 3 and 5 and it is possible that the error of mistaking a polymorphous cell sarcoma for a malignant form of myoblastoma may have been committed. It may, however, be pointed out that the histological picture closely approximates that shown by Figure 3 of the published article of Horn and Stout [8] and the description accompanying their case 2.

The essential criteria for a malignant tumor have been accepted as some or all of the following: (1) Hyperplasia of cells beyond that ordinarily seen in an inflammatory process; (2) an atypical quality of tumor cells—anaplasia; and (3) displacement or transportation of tumor cells from the place of their origin. The first three tumors reported here, besides exhibiting the microscopic features of a granular cell myoblastoma, showed evidence of a progressive growth with a spread along the lymph channels and a clinically malignant course. It is necessary to admit that no reasonable explanation can be offered for the unduly large number of cases in this series showing malignant evolution, and it is for this very reason that I had the temerity to add to the literature on the subject. It may be suggested that, though a majority of these tumors evolve slowly and show a benign course, others may grow rapidly, spread along lymphatics, and give rise to wide dissemination with a fatal termination. Experimental work on cancer has tended to establish that "Malignancy is a universal cell potentiality in that any cell has inherent in its makeup the potentiality for unlimited or uncontrolled growth." [10] The differences of opinion concern the relative incidence of malignancy in tumors of this type and whether the malignant tumors should be grouped with polymorphous cell sarcomas, rhabdomyosarcomas, or the malignant variety of granular cell myoblastoma. The competence of cells to undergo malignant proliferation is a property which cannot necessarily be correlated with

their histological features. This was particularly noticeable in some of the cases described above, as it was bewildering to follow a relentless clinical course coupled with a relatively innocent histological appearance. "No morphologic terms and characteristics can serve to indicate the developmental potencies of cells." [7]

4. The occurrence of granular myoblastomas in localities where striped muscle is not normally found has been explained on the basis of a close embryologic association of the precursors of the skin, the muscle and the bone; or a displaced persistence of embryonic cell rests. The work of experimental morphologists makes it unnecessary to seek for such cell rests in the genesis of most neoplasms. There is accumulating evidence to show that birth does not abolish the developmental potentialities of cells along certain specific directions. The mesenchymal cells in the vicinity of small blood vessels are "endowed with all potencies of embryonic mesenchyme." It is conceivable that mesenchymal cells may take on a neoplastic growth under appropriate stimuli and that the cells may assume a structure along the direction of their formative potencies. The structure of the resulting new growth may not necessarily resemble closely the structure and arrangement of cells possessing such potencies. It therefore seems unprofitable to assume the existence of embryonic cell rests at the sites of origin of the last three tumors (cases 4, 5, and 6).

Case 1 was referred to me by Prof. W. A. G. Karunaratne; case 5, by Dr. P. H. Kronenberger; and case 6, by Dr. H. D. Laemmle. The autopsy on case 3 was performed by Maj. P. V. Gharpure, who sent gross and histological material for study. The valuable criticism of Prof. A. P. Stout has been of great assistance in the preparation of this paper.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

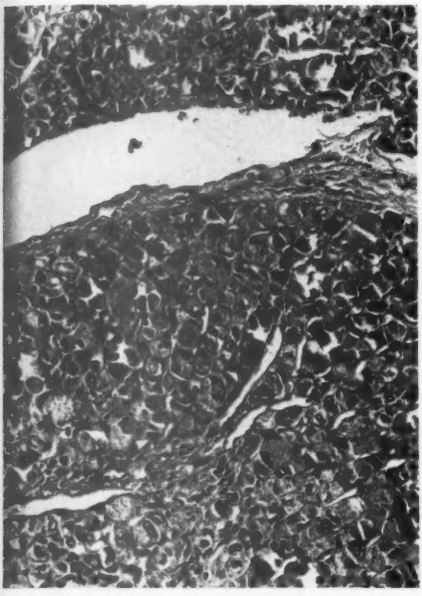
PLATE 117

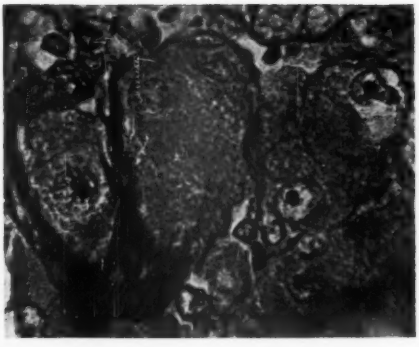
FIG. 1. Case 1. Sheets of large polyhedral cells are interspersed with thin-walled blood capillaries. Hematoxylin and eosin stain. × 170.

FIG. 2. Case 1. Three large polyhedral cells with characteristic granules are shown at higher magnification. Masson's trichrome stain. × 750.

FIG. 3. Case 1. A delicate reticular stroma surrounds individual cells and small groups. Gomori's silver impregnation stain. × 170.

FIG. 4. Case 3. Transverse section of the heart, showing the inferior half. The tumor nodules in the ventricular walls and the papillary muscles are clearly seen.





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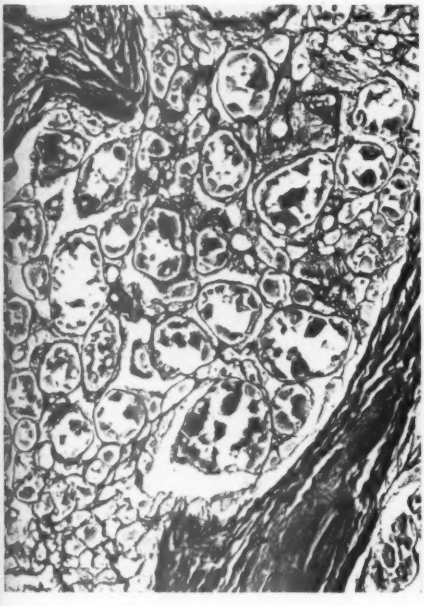

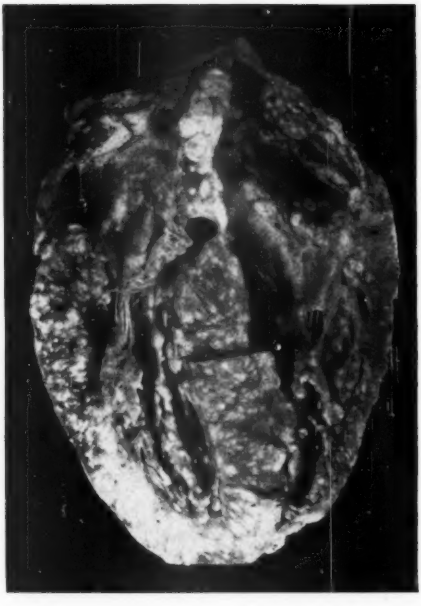

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PLATE 118

FIG. 5. Case 3. Tumor deposits in the heart muscle. The cardiac muscle fibers are seen as a darker band between two deposits. Hematoxylin and eosin stain. × 130.

FIG. 6. Case 4. Lateral view of the skull, showing an area of destruction at the parieto-occipital junction.

FIG. 7. Case 4. The character of the granular cells, the details of the nuclear structure, and the intercellular reticulum are shown. Masson's trichrome stain. × 750.

FIG. 8. Case 4. Sheets of tumor cells with an attempt at an organoid pattern. Hematoxylin and eosin stain. × 170.

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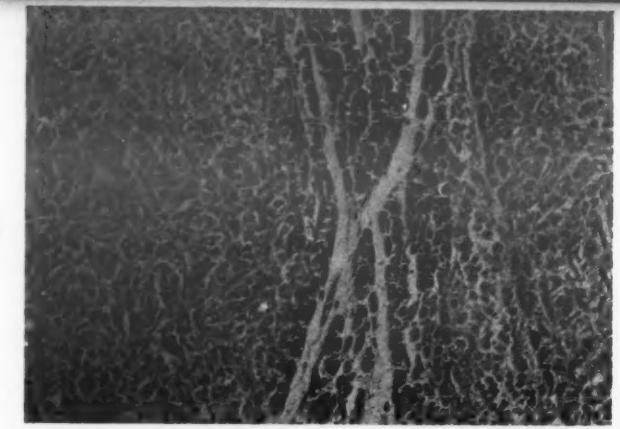

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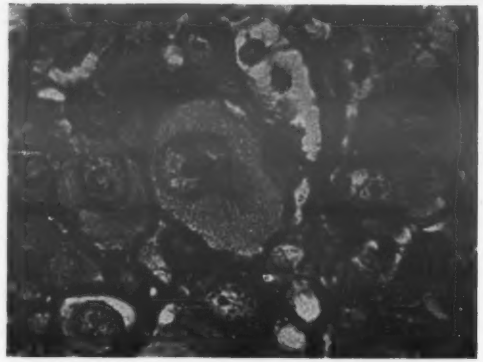

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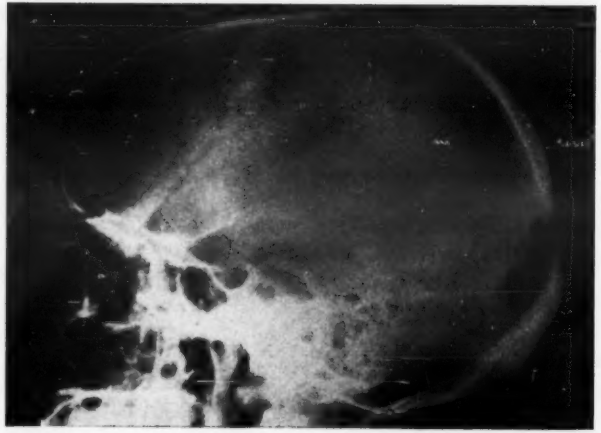

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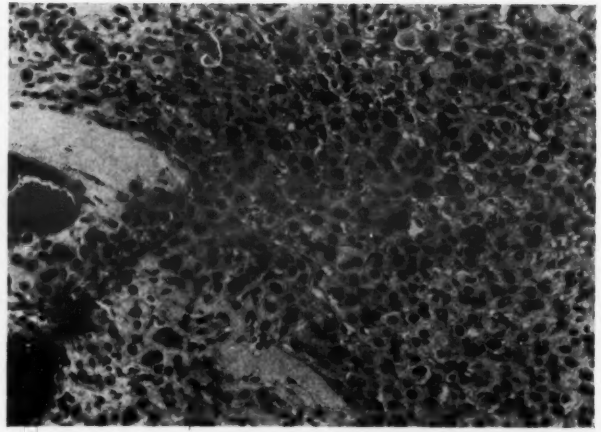

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Granular Cell Myoblastoma

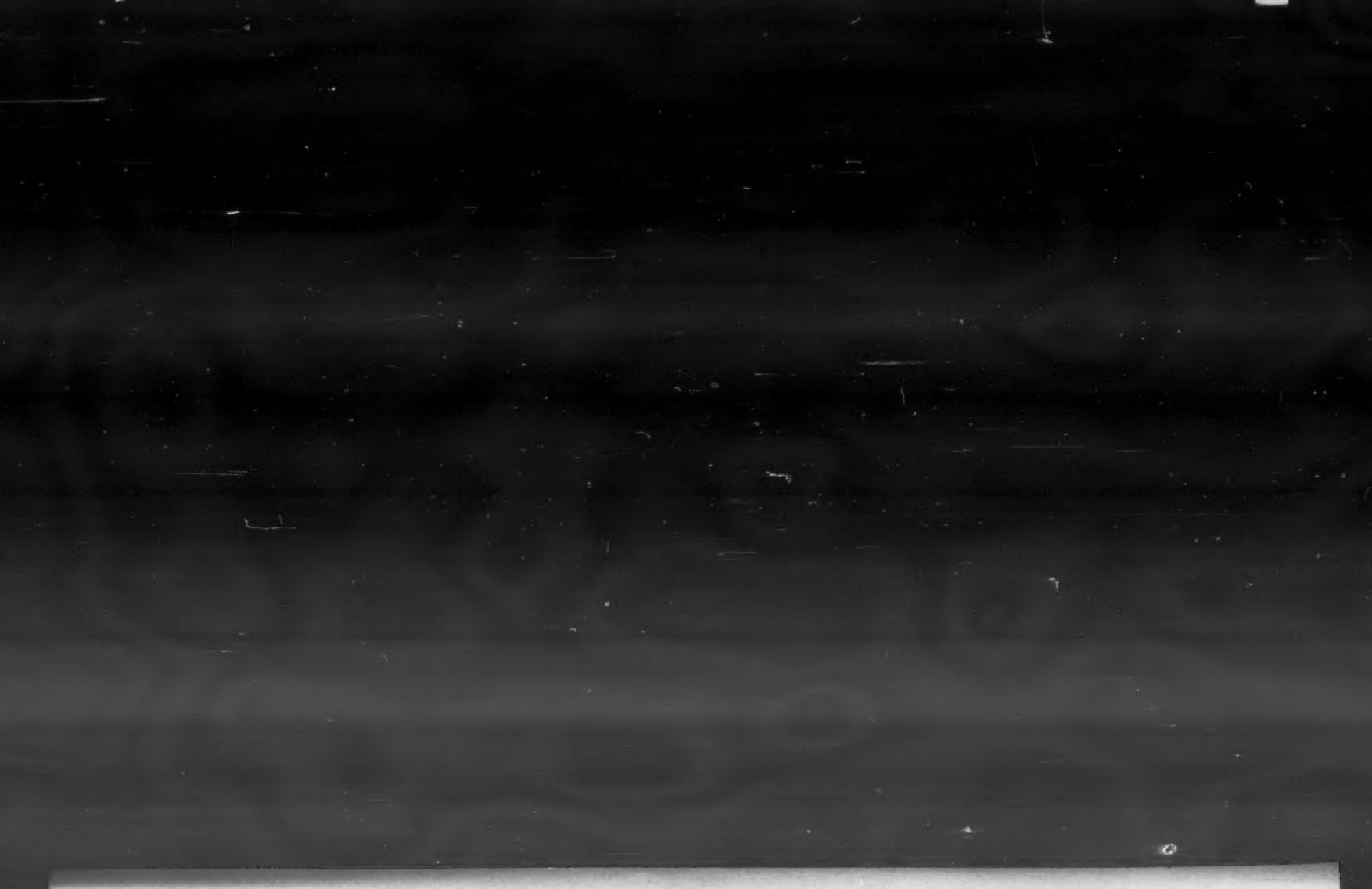
PLATE 119

FIG. 9. Case 5. Lateral view of the skull, showing destruction of bone in the squama temporalis and in the posterior portion of the left mastoid region.

FIG. 10. Case 5. Groups and rows of polygonal, cylindrical, and fusiform tumor cells. Hematoxylin and eosin stain. × 170.

FIG. 11. Case 6. Ribbon-like cells contain granular cytoplasm. Hematoxylin and eosin stain. × 750.

FIG. 12. Case 6. Photomicrograph showing the character of the tumor cells and the rich fibrovascular stroma. Hematoxylin and eosin stain. × 170.



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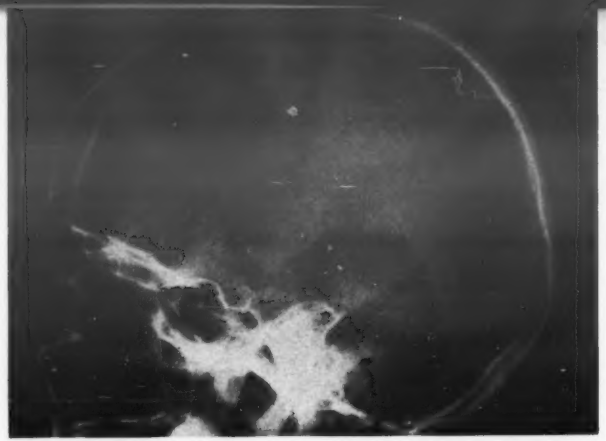

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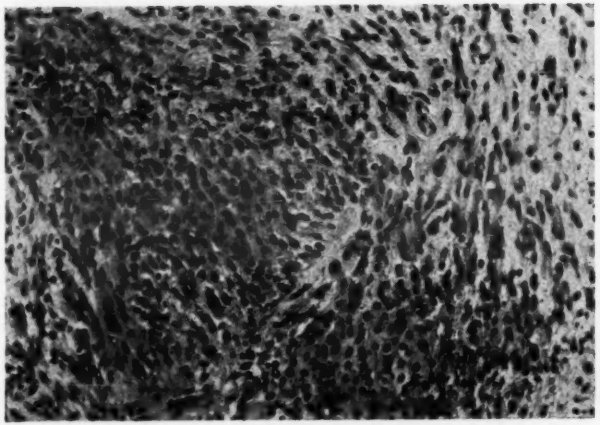

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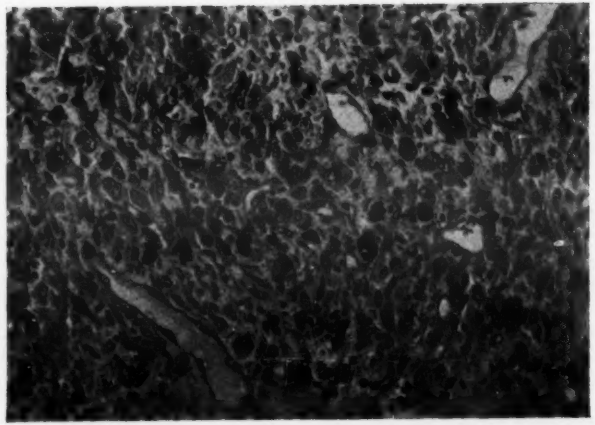

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Granular Cell Myoblastoma

SUBCORTICAL FIBROBLASTOMA OF THE BRAIN

A Case Report *

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Before describing a case of subcortical fibroblastoma of the brain, it is thought advisable to summarize the numerous and often conflicting views pertaining to the origin of such tumors. Different authors have formulated different theories, or have demonstrated seemingly convincing proofs, relating to the derivation of certain tumors of the central nervous system. Each has proposed what seemed to him to be the most appropriate name to characterize adequately the nature of the growth. The term dural endothelioma was frequently employed until Mallory [1] showed that the type cell of these tumors was the fibroblast of the arachnoid membrane. It logically followed that they received the name arachnoidal fibroblastoma. Cushing,[2] however, preferred the more inclusive term meningioma, which Learmonth [3] modified to leptomeningioma, thereby excluding the possible suggestion of dural origin. The name meningeal fibroblastoma advanced by Penfield [4] is, however, more of a blanket expression than Mallory's. In one article, Alpers, Yaskin, and Grant [5] use only the simple fibroblastoma designation, while in another report,[6] in which 75 such tumors are analyzed from several standpoints, the term meningeal fibroblastoma is employed. In reporting one of Mallory's specimens, Bailey [7] also called it simply fibroblastoma. In order to express the origin of the growth, Elsberg [8] preferred Penfield's designation of meningeal fibroblastoma.

Globus [9] classified the meningiomas from phylogenetic and ontogenetic points of view, and emphasized that fibroblastoma is not descriptive of the majority of meningiomas and that it can be applied only to a small subgroup. He proposed subdivision into five types based on origin and structure: (1) Meningioma indifferentiale, mesenchymatous meningioma; (2) Meningioma omniforme, primitive meningioma; (3) Pachymeningioma, fibroblastic meningioma or dural fibroblastoma; (4) Leptomeningioma, arachnoid (?) meningioma; (5) Meningioma piale, pial (vascular) meningioma. He conceded, however, that "the fibroblastic character is not denied for many of the cellular elements in a large number of meningeal tumors nor for the dominant cell type of a few."

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As long as there is general agreement among all the authors that these tumors arise in the meninges, whether on the surface or in the prolongations carried by the blood vessels into the deeper layers of the parenchyma, it seems that the nature of the growth is adequately characterized by the designation of the cell type of which it is composed, regardless of the original location of the cell, provided that this is accepted as a fibroblast. The difference of opinion, indeed, pertains rather to the type of meningeal membrane than to the type of cell.

While many fibroblastomas with or without qualifying adjectives have been reported in the literature, most of those tumors were surface growths. That is, although they pressed deeply into the brain substance, they were at one place attached to the overlying dura. According to Babcock,[10] "Meningiomas constitute one-eighth of all brain tumors." Elsberg [8] stated that "probably 90 per cent of meningeal growths are fibroblastomas." Kazan, Weller, and Jaramillo [11] observed that "it is commonly stated that meningiomas comprise some 15 per cent of all forms of brain tumor" and that of 431 cases of brain tumors found at autopsy, 74 (17.1 per cent) were meningiomas.

Up to 1932, according to Alpers, Yaskin, and Grant,[5] only three fibroblastomas were known to have been reported that were subcortical and without surface connection. However, another deep intracerebral fibroblastoma was described by Petit-Dutaillis and Bertrand,[12] and Baker and Adams [13] reported a subcortical tumor in the right middle and inferior frontal convolutions which, although not encapsulated, was well demarcated from the surrounding brain tissue. Histologically, it consisted of numerous fine and coarse strands of intertwining collagenous fibers with a moderate number of cells interspersed. The authors identified the cells as fibroblasts and called the tumor a fibroblastoma. These five so-called intracerebral fibroblastomas were in the brain substance. Similar growths of another variety which are intraventricular and originate probably in the choroid villi are not included in this group. Such tumors were described by Gardner and Turner [14] who give the pertinent references.

As a closely related finding, a case of subcortical meningioma of the cerebellum was reported by Christophe and Divry,[15] who quoted the histological description of the growth by Harvey Cushing, whose opinion they had obtained: "Case M. . . . proves to be a typical meningioma and although intracerebellar subcortical meningiomas are so rare that we have no definite example in our series of 315 cases, nevertheless there is no reason why it cannot occur. Just where it has taken its origin is difficult to surmise, but I would suppose that it might have come off from the chorioid plexus or from the tela chorioidea superior

(velum interpositum) which have been described as [sources of] subcortical meningiomas of cerebral hemispheres without dural attachments."

In an attempt to differentiate these tumors from those that invade the brain substance from its surface, they were named primary fibroblastomas. This should not be misinterpreted, however, to mean that such a tumor is a first focus that may give rise to secondary growths elsewhere. These tumors *do not* metastasize. Indeed, one of their chief characteristics is that they are completely encapsulated and more or less easily shelled out from their nests; and, in spite of a microscopical structure that strongly resembles fibrosarcoma, they are classified as nonmalignant tumors. From the clinical viewpoint, because of their location and size, these neoplasms produce severe symptoms, depending upon the nature of the brain tissue which is affected. Many were causes of death, directly or indirectly.

Report of Case

The record accompanying the part of a tumor that was sent to this Unit by the Veterans Administration Hospital, Bedford, Massachusetts, may be abstracted as follows:

The patient was a male, 54 years old, who was admitted on July 6, 1945, and was diagnosed as having a psychosis with syphilitic meningo-encephalitis, and right hemiparesis, on August 24, 1945. A metastatic carcinoma was considered upon admission, apparently because of the right hemiplegia and loss of weight. There had been hemorrhage from the stomach and melena in April, 1944. Because of his mental state, which was marked chiefly by loss of appetite, inaccessibility, and broodiness, this patient had received seven electroshock treatments with temporary benefit.

The patient had gross hematuria on July 14, 1945. The spinal fluid on July 17, 1945, was clear; globulin, large amount; cells, 6; total protein, 150 mg.; Wassermann reaction, suggestively positive in 0.5 cc., positive in 1.0 cc.; colloidal gold test, 4433321000.

On October 8, 1945, the patient had a temperature of 103°F. The clinical impression was that of advanced carcinoma. He received penicillin, as well as soluble vitamin B, twice daily. On November 2, 1945, there was edema of the right forearm and hand, with blebs; pulsations in the arm were good. The patient expired the next day.

Clinical Diagnoses. Right hemiparesis, possible peptic ulcer, decubital ulcer over sacrum.

AUTOPSY FINDINGS

The post-mortem examination (I. J. B.) was performed at the Veterans Administration Hospital, Bedford, Massachusetts. The body was markedly undernourished. The right forearm showed denuding of the skin in large patches, approximately the size of the palm of the hand. The skin was bluish. There was a sharp line of demarcation approximately 1 inch above the cubital fossa. The right hand, itself, was swol-

len. There were several blisters upon the back of the right hand, the largest approximately 1 inch in diameter. Within the blisters, a clear fluid was found. Dissection of the cubital fossa disclosed that practically all of the veins were occluded. The thrombotic material appeared to be very fresh. Within the tissue a serous fluid was found. The arteries appeared normal. A healing excoriation was found at the right elbow, upon the dorsal surface. The subcutaneous tissues appeared to be edematous in this region. No evidence of gas formation was found within the tissues themselves.

There was a large decubital ulcer at the base of the spine, measuring approximately 4 by 3 inches. The subcutaneous tissue in this area appeared somewhat necrotic. Several ulcerations of the posterior surface of the right lower extremity were found also. One measured approximately 4 inches, the other approximately 3 inches in length, and each approximately 1 inch in width.

Head. Scalp and skull appeared normal. The surface of the brain showed no abnormality. Section of the brain disclosed a tumorous mass, 2½ by 1¾ by 1½ inches, in the left frontal lobe. This mass was rather firm and pale pink. It appeared to be encapsulated and shelled easily out of the adjacent brain tissue. It also appeared to be somewhat lobulated. The rest of the brain showed no abnormality. The basal vessels showed no arteriosclerosis. The base of the skull appeared normal. The brain itself weighed 1420 gm.

Thorax. The left lung weighed 390, the right, 570 gm. The left lung showed adhesions to both the lateral and posterior walls of the chest. However, on section both lungs appeared normal. The heart was normal in size and shape and its musculature and valves were normal. The aorta showed no lesions. The coronaries were patent and normal throughout.

Abdomen. The intestines appeared normal, as did the stomach and pancreas. The liver was normal in size and shape. The adrenals were normal. The left kidney weighed 150, the right, 110 gm. The capsules stripped with ease. Sections of both kidneys were normal. The abdominal aorta was normal grossly. The spleen weighed 100 gm. and its surface was slate-colored. The section was dark red and fairly firm, with many whitish strands strewn throughout the splenic pulp. The gallbladder showed no abnormality.

Male Pelvis. No abnormal findings were noted in the pelvis.

Pathological Diagnoses (*Including Histological Findings*). Tumor of brain, left frontal lobe (meningeal fibroma); thrombophlebitis, with moist gangrene, right forearm; adhesive pleurisy, left lung.

Gross Description *

The specimen was a grayish white lump of tissue, one side of which was flat where it had been cut before it was sent to this laboratory. The cut surface was roughly triangular with rounded corners. Opposite the cut surface the tissue was rounded, somewhat nodular, and encased in a thin capsule. At one side of the triangular surface there were fragments of connective tissue bands which gave the impression that it was there that the tumor had been attached to the surrounding structures and had been severed surgically. The dimensions were approximately 32 by 25 by 22 mm. for the three main diameters. The nodule had a firm consistency, with the elasticity of a medium-hard rubber eraser. The cut surface showed very light gray granular areas which were confluent and occupied the greater part of the cut surface. Between these, the tissue appeared firmer and more like connective tissue, and here there were many small, dark spots ranging in size from that of a pin-point to that of a millet seed, evidently transverse sections of blood vessels or minute hemorrhages.

Microscopical Description *

Some of the sections were stained with hematoxylin and eosin, others were impregnated with silver by Perdrau's method.

There was a strong capsule, consisting mainly of connective tissue fibers of collagenous character. Branches of the connective tissue bundles formed a dense network with an abundance of the cellular elements. The cells of the matrix varied in size, shape, and structure. Some had round or oval, large, lightly stained vesicular nuclei in which there was a fine keratin network, at the intersections of which there were small chromatin granules. Other cells had small, darkly stained nuclei. The cytoplasm was usually elongated, fusiform, and stained fairly homogeneously with eosin. From the periphery both fibrous and cellular elements proceeded into the deeper layers. The cells formed dense, solid cords and masses which spread in an irregular pattern in all directions. While they were richly cellular, no purposeful arrangement or attempt to form some differentiated structure could be observed. Indeed, the polarity of the cells was completely upset. In some areas there were massive cell groups without discernible connective tissue fibers, strongly resembling malignant growths of mesodermal origin. Disseminated among the other cells were many giant cells with several nuclei which were closely packed at the central portion of the cytoplasm. The cells forming the cords and masses varied from large

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oval elements with large vesicular nuclei, to small cells with dark pyknotic nuclei.

There were several areas in which the cells assumed a spiral arrangement around either a small blood vessel or a homogeneously stained acidophilic lump which seemed to be composed of coarse bands resembling a loose skein. Some of these cell groups formed conspicuous whorls of various sizes. The tendency of the cell masses to encircle foci of other elements was very prevalent.

Perdrau's silver impregnation method brought out large bundles of collagenous fibers composed of coarse and fine strands, as well as very delicate reticulin, the filaments of which formed a complex network. The fibers surrounded the blood vessels in a massive, dense skein and then branched off into a gradually looser network. The capsule itself was composed of fibrous elements of the same type.

The whole tissue was richly supplied with blood vessels ranging from approximately 500 μ in diameter to a network of dense capillaries. All blood vessels were filled to capacity with red blood cells. There were several whose walls showed evidence of a break-through by adjacent tumor masses. There, the invading cells could be seen in the lumina.

While no definitely characteristic mitotic figures were identified, there were many extremely dense, darkly stained elements which perhaps represented phases of rapid cell division.

Against the microscopical structure, which is strongly suggestive of a malignant tumor, stand the gross findings of a growth which was encapsulated and easily separated from the adjacent brain substance. Considering both the gross appearance and the microscopical structure, a diagnosis was made of subcortical fibroblastoma of the brain.

Summary

1. Dural endothelioma, arachnoidal fibroblastoma, meningioma, leptomeningioma, meningeal fibroblastoma, and fibroblastoma of the brain are synonymous terms used by different authors to designate the same tumor which is comparatively common as a surface growth.

2. The so-called primary or subcortical variety of this neoplasm is very rare. An example of subcortical fibroblastoma, which was found at autopsy, is the basis for this report.

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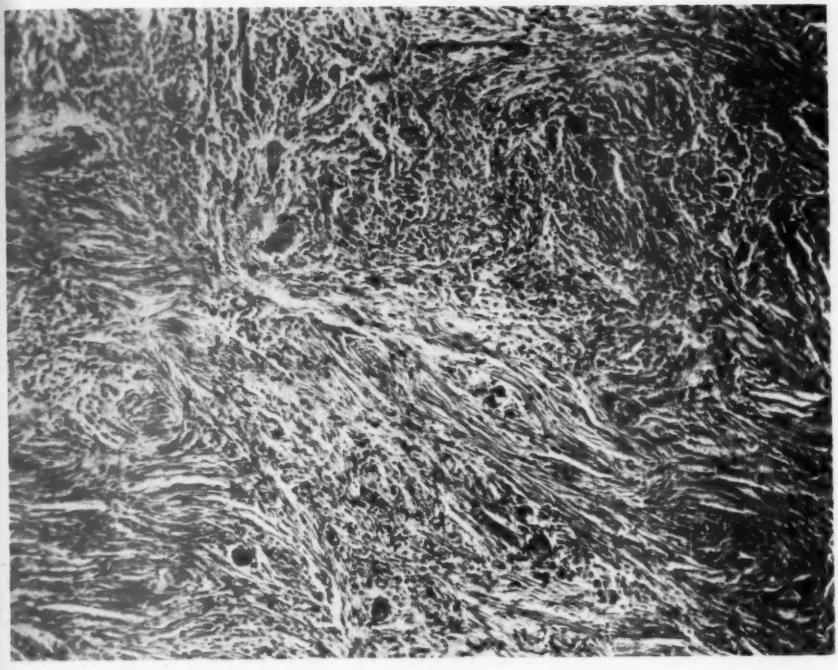
[*Illustrations follow*]

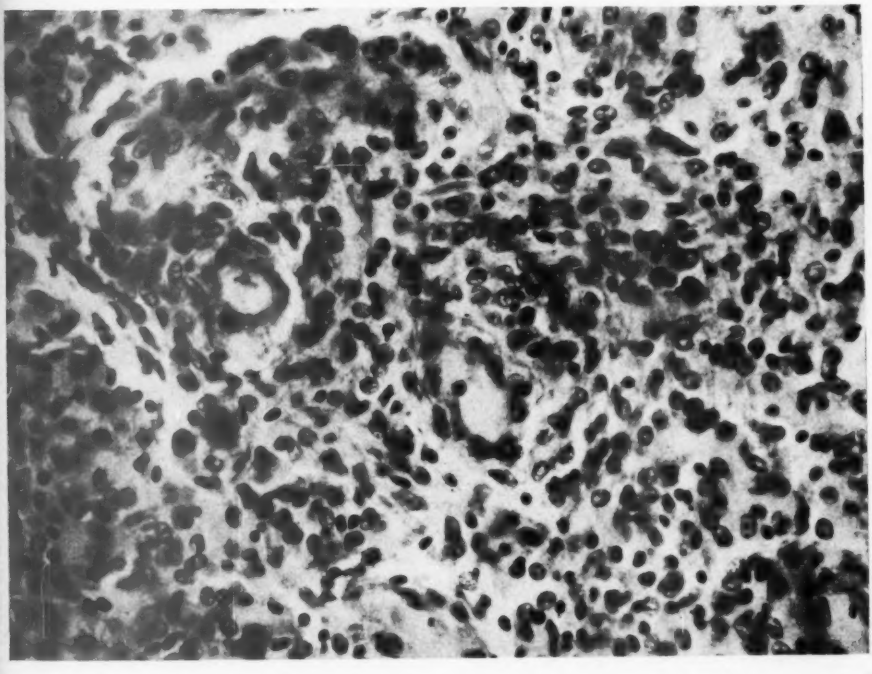
DESCRIPTION OF PLATES

PLATE 120

FIG. 1. Cellular and fibrous elements in approximately equal proportions. Giant cells. Hematoxylin and eosin stain. × 100.

FIG. 2. Cellular area showing a great variety of nuclei and masses without formation of differentiated structures. Hematoxylin and eosin stain. × 100.



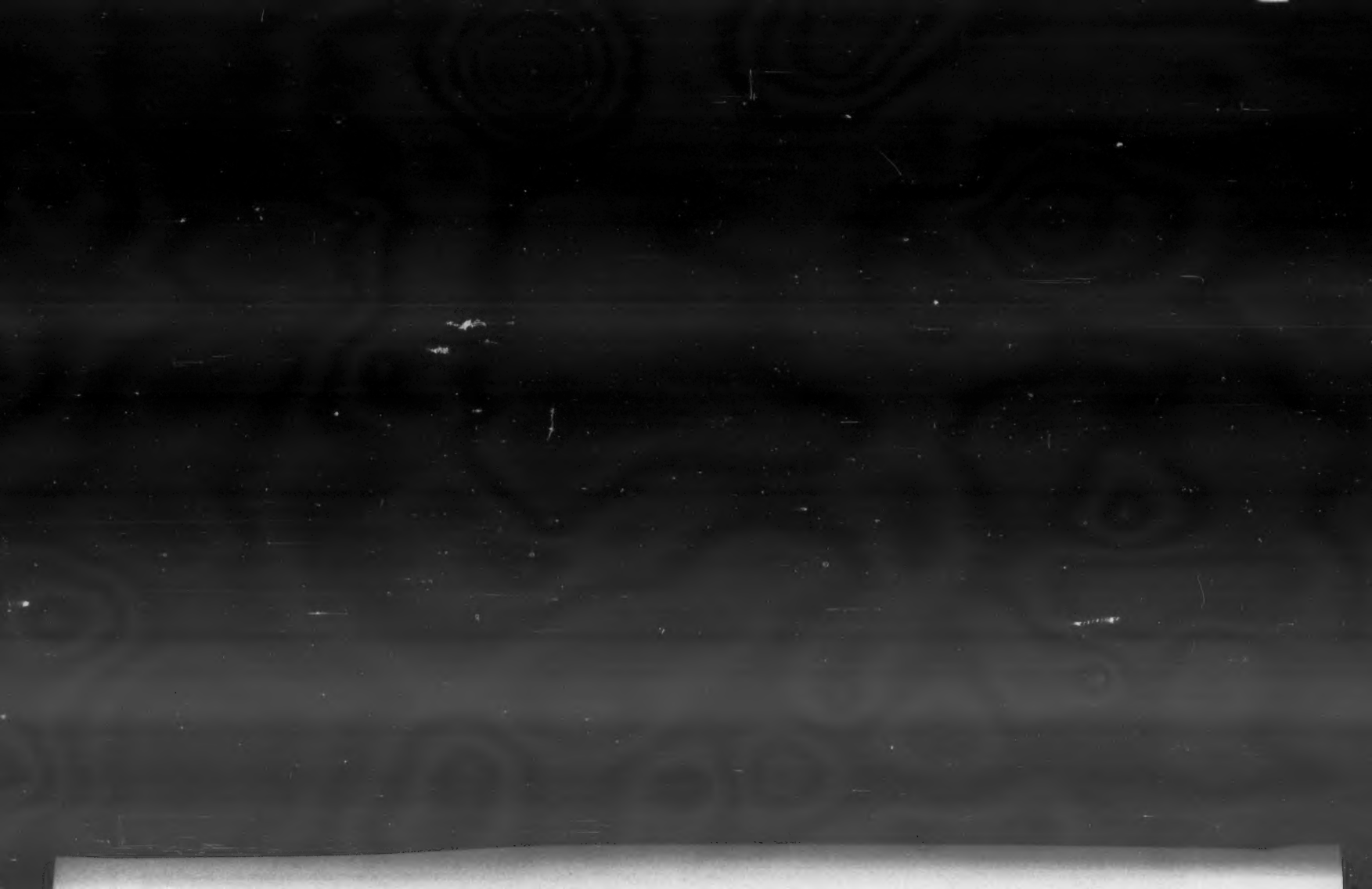


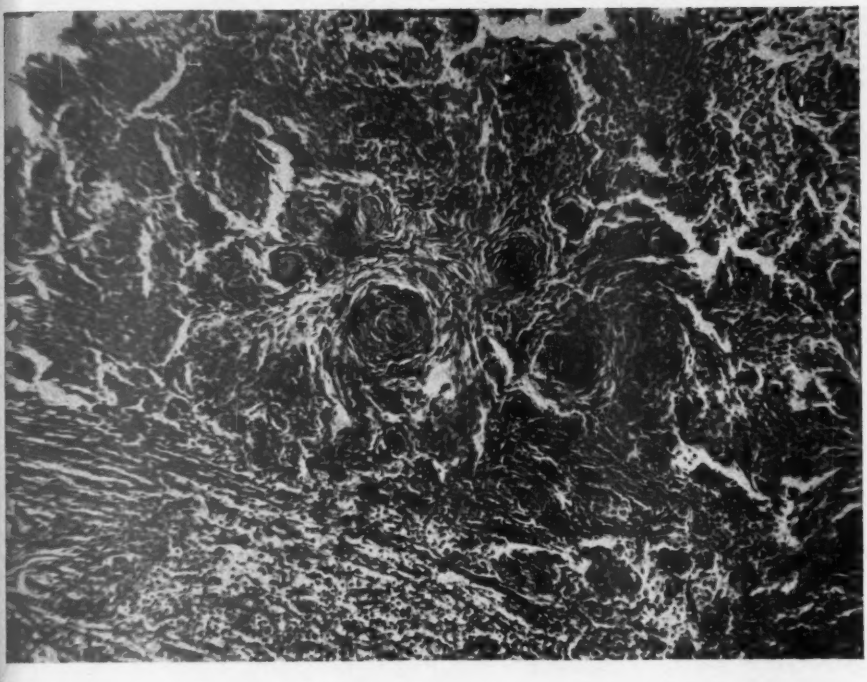
Groszberg and Blumenthal Subcortical Fibroblastoma of the Brain

PLATE 121

FIG. 3. Whorl formation. Hematoxylin and eosin stain. × 100.

FIG. 4. The center whorl of Figure 3. Hematoxylin and eosin stain. × 430.





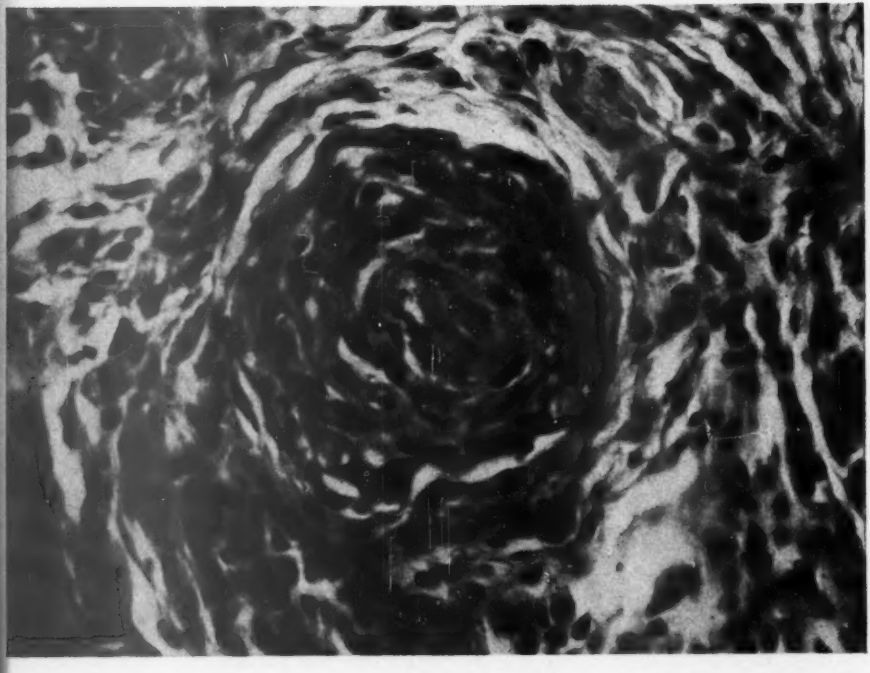


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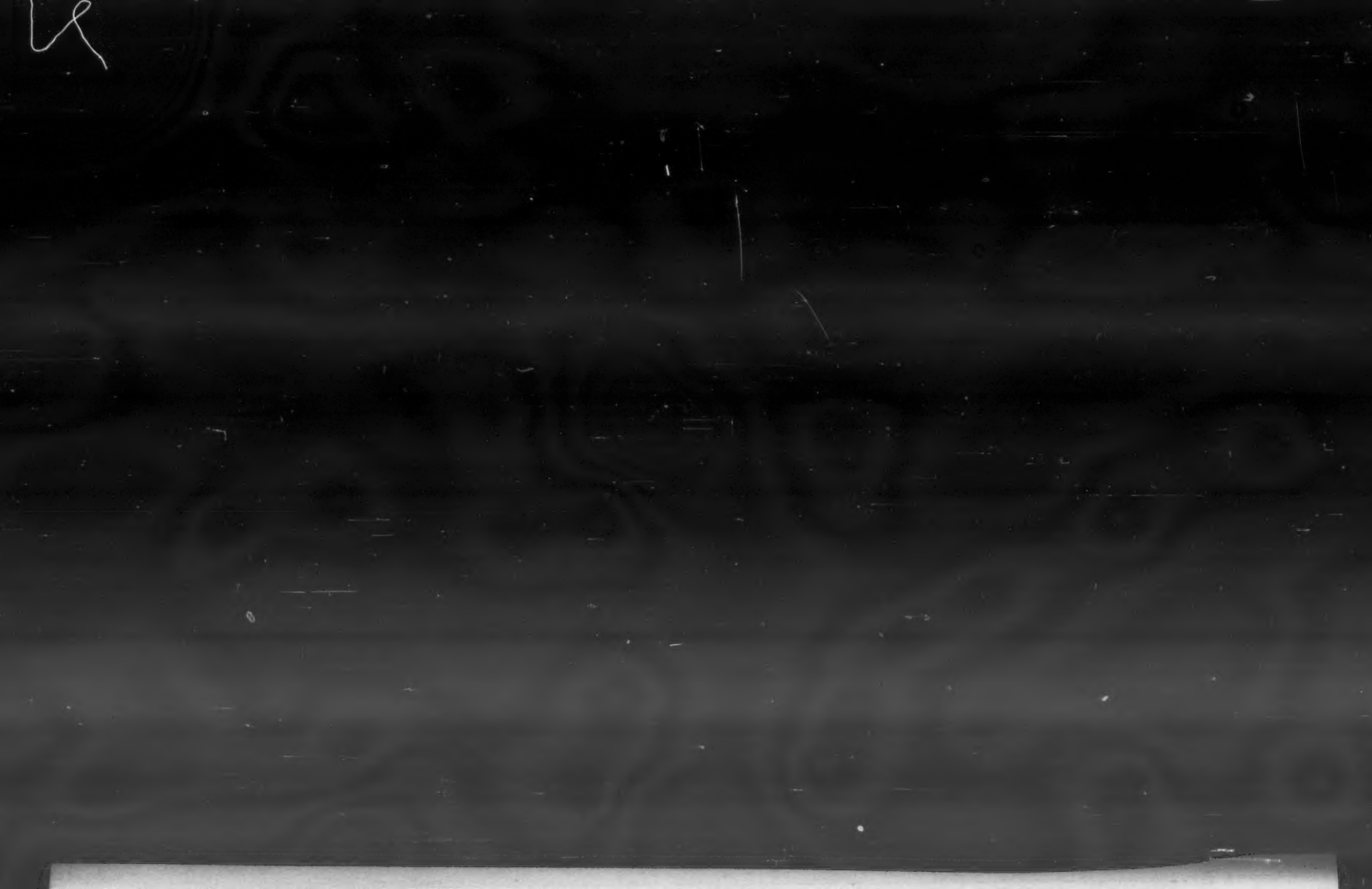
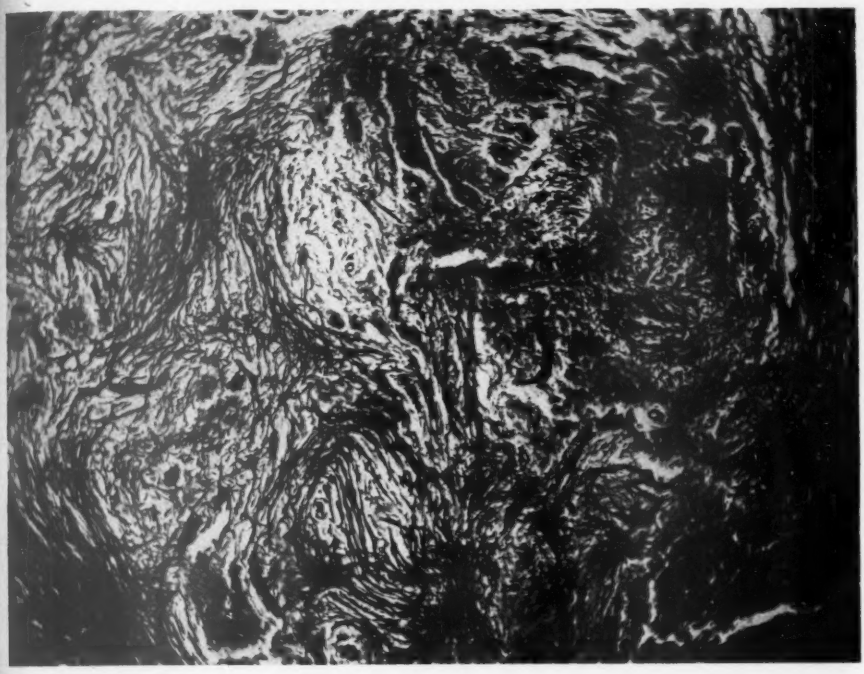
PLATE 122

FIG. 5. Bundles of coarse and fine collagenous fibers. Perdrau's silver impregnation. × 100.

FIG. 6. The center portion of Figure 5. Perdrau's silver impregnation. × 430.





Groszberg and Blumenthal Subcortical Fibroblastoma of the Brain

OSTEOPETROSIS: ALBERS-SCHÖNBERG DISEASE (MARBLE BONES)

Report of a Case and Morphologic Study *

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The purpose of this paper is to present a brief clinical history and a detailed pathologic description of a case of marble bones.

In 1904 Albers-Schönberg [1] published a clinico-radiologic study of a case of generalized osteosclerosis. He was able to differentiate this case from a group of apparently similar cases of osteosclerosis associated with primary blood dyscrasias previously reported by other authors. This condition was characterized by increased density of the cortical and medullary portions of the entire osseous system, anemia, and enlargement of the liver, spleen, and lymph nodes. The homogeneous roentgenologic appearance of the bones suggested the name of marble bones (maladie des os marmoreens, Marmorknochenkrankheit, Marmorskelett, morbo marmorea). In 1907 Assmann [2] reported 4 such cases. He conjectured that since this condition appeared to be primarily a disease of the blood-forming organs, osteosclerotic anemia (osteosklerotische Anemie) would be a more appropriate name. Laurell and Wallgren [3] were impressed by the multiple pathologic fractures which occurred in these cases and they introduced the term osteosclerosis fragilis generalisata. In 1926 Karshner [4] collected 18 cases of marble bones from the literature and added 4 of his own. The petrified nature of the bones suggested the term osteopetrosis (stony bone). In 1930 Pirie [5] collected 26 cases from the literature and described 5 new cases. In his cases a drill sank into the bony substance as it would into a mass of chalk. Inasmuch as roentgenologic investigations showed that these bones were of the same density as ordinary chalk, Pirie proposed the name chalky bone for the disease. Among other terms used are lime gout, and congenital osteosclerosis (angeborene Osteosklerose). In 1941 Higinbotham and Alexander [6] collected 131 cases from the literature and added 4 cases. To date 148 cases have been reported.

Many of the cases described cannot be regarded as authentic instances of Albers-Schönberg disease. The clinico-radiologic criteria used to diagnose the condition do not entirely differentiate osteopetrosis from similar, though unrelated, disease entities. A large number of the reported cases are in reality instances of primary disease of the blood with secondary osteosclerosis and myelofibrosis. Zwerg and Laubmann [7] recognized only 55 of the reported cases as instances of

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true marble bones. They predicated their conclusions on the following triad of changes: osteosclerosis of the entire skeleton, spontaneous bone fractures, and morphologic alterations in the peripheral blood. However, this triad can be found also in diseases other than marble bones. Further investigation seems necessary for more definite diagnostic criteria.

The only constant diagnostic feature of osteopetrosis is the abnormal increase in the hardness and density of the bones of the skeleton. This, however, is not characteristic of Albers-Schönberg disease alone; it may be seen as a rare form of osteodystrophy associated with other conditions. Heuck [8] was the first to describe a case of generalized osteosclerosis associated with leukemia. Subsequently Neumann,[9] von Jaksch,[10] and others reported similar cases. Jordan and Scott [11] grouped the cases of osseous sclerosis accompanying primary blood dyscrasias according to the alteration of the cells of the peripheral blood. Generalized osteosclerosis has also been found in rare instances of chronic poisoning with fluorine, phosphorus, and strontium. More rarely, it is seen in cases of generalized metastasis of carcinoma to the skeleton.

Secondary anemia is commonly seen in marble bones. Usually it is of a hypochromic, myelophthisic nature, although it may be hyperchromic. The peripheral blood shows immature red blood cells, chiefly normoblasts, and occasional megaloblasts. The platelet count may be normal or low. In children the leukocyte count is high but it may be low in adults. In severe cases of anemia there is a tendency to bleed from the skin and mucous membranes.

Some of the findings are not always constant. The infant or juvenile patient is frail and underdeveloped. The epiphyses appear at the normal time or are slightly delayed and the epiphyseal lines are slow in closing. The growth of the bones in length appears to be normal. The fontanelles remain open longer than usual. Walking, talking, and dentition are delayed. The children appear older than their actual age. The skin is dry, the root of the nose is indented. There are often seen multiple spontaneous bone fractures which produce deformities. Frequently some of the following conditions may be found: Frontal bossing, craniotabes, enlargement of the skull, coxa vara, dorsal scoliosis, beading of the costochondral junctions, deformities of the ribs and chest, enlarged epiphyses of the long bones, bowlegs, syndactylism, achondroplasia, rachitic or pseudorachitic changes, imperfect dentition, overgrowth of the lower jaw, and osteomyelitis of the jaw or of other bones.

Some of the younger patients present symptoms caused by narrow-

ing of the different foramina of the skull. Stenosis of the optic foramina may produce optic atrophy, nystagmus, and blindness. Hydrocephalus and subarachnoid hemorrhages are caused by obstruction of the venous flow at the cranial foramina. Various otologic changes may result from impingement on the auditory nerves.

The signs and symptoms of marble bone disease vary in severity and in time of appearance. They may appear early or late in the course of the disease and may be mild or severe in character. In the infantile and juvenile cases the course is usually more severe and often rapidly fatal. Death may result from anemia, hemorrhage, intercurrent infections, or toxemia secondary to chronic suppurative foci in the bones. In the post-adolescent and adult forms the course is relatively slow and benign. The disease may be carried into adult life without clinical evidence of its presence; it is often detected incidentally after roentgenologic examination of the skeleton. More rarely, attention is called to it by the changes in the configuration of the skull or limbs.

Roentgenologically, the bones of the entire skeleton are homogeneously opaque. The vertebrae, the central part of the pelvis, the base of the skull, the proximal portion of the femur, and the distal ends of the tibia and fibula are more severely affected. The trabecular structure of the bones is partially or completely obliterated and the medullary canal may be reduced in size or absent. The distal ends of the shafts of the long bones and the centers of the flat bones are less sclerotic than the remainder of the skeleton. As a rule, the bones are of normal shape and length but there may be some alteration in their contour. Frequently there is clubbing of the ends of the long bones, especially of the proximal portion of the humerus and the distal portion of the femur. Roentgenologic evidence of rickets may be present in some infants (Karshner,[4] Kudrjawtzewa,[12] Hässler and Krauspe,[13] Kramer and Halpert,[14] McCune and Bradley [15]). Transverse bands of less dense bone running parallel to the epiphyseal plates may be seen in the metaphysis of the long bones and in the short bones. The clinoid processes of the skull are clubbed and thickened. The sella turcica is shallower than normal and the pneumatic structures appear dense.

Systematic chemical analyses of the osseous system in marble bones have been rare. McCune and Bradley [15] were able to find only 5 cases in the literature in which the chemical composition of the bones was analyzed. Metabolic studies of calcium and phosphorus were incomplete. In 1939 Kramer, Yuska, and Steiner [16] reported a case of osteopetrosis in which a comprehensive chemical investigation of the bones was made. Pincus, Gittleman, and Kramer [17] have made similar study of the bones in the case which will be described in this paper. The

conclusions drawn from the study of the above 2 cases are briefly as follows: The calcium and phosphorus content of the bone ash in osteopetrosis is distinctly higher than in normal infants of corresponding age, except in the flat bones of the skull. Calcium and phosphorus are present in the form of tertiary calcium phosphate except near the epiphyses of some of the long bones where a calcium phosphate compound of lower molecular ratio is found. There is diffuse hypermineralization of the bones and a lowered molecular ratio of tertiary calcium phosphate to calcium carbonate, indicating a relative increase in the latter.

Incidence. Osteopetrosis may begin in utero as soon as the process of ossification begins. Pirie [5,18] diagnosed the condition in a case before birth because of the increased density of the bones of the fetus as seen on roentgenologic examination and from the knowledge that other living members of the family suffered from marble bones. The benign form of the disease may begin in early life and may not manifest itself clinically until late. Gortan [19] reported a case of marble bones in a female who lived until 72 years of age. The more typical forms of the disease are seen in infants and juvenile patients. The majority of the cases occur in patients under 10 years of age. Judging from the anamneses, it may be said that some stillbirths and miscarriages may be accounted for by the prenatal fatal form of this disease. There appears to be a slight preponderance in males.

Etiology. There is no satisfactory explanation for the causation of marble bones. Constitution and heredity appear to play a prominent rôle. Many of the cases give a familial history of the disease or a history of parental consanguinity. Sick [20] reported 3 siblings and one cousin who had osteopetrosis; McPeak [21] mentioned 8 cases in 3 generations of one family. In our case, and in other reported instances, the parents were first cousins. Nine of the 40 cases collected by Orel [22] showed parental blood relationship. In a review of 121 cases from the literature van Creveld and Heybroek [23] found that 49 patients gave a familial history of osteopetrosis or of parental consanguinity. This led Kudrjawtzewa [12] and others to conclude that the disease is initiated by a mutation of the germ plasm. The high incidence among relatives suggests the existence of a hereditary factor which is carried as a recessive mendelian character. Some authors suggest that the fatal form of the disease may be carried as a mendelian dominant rather than as a recessive factor.

The cause of Albers-Schönberg disease is still obscure. In the case described by Albers-Schönberg, the patient suffered from lues; only 2 other cases with lues have been reported. Pirie [5,18] believed that Albers-

Schönberg disease is the end-result of an acute, spreading epiphysitis. Laurell and Wallgren,[3] Sick,[20] Lorey and Reye,[24] and others believed that it is due to an imbalanced distribution of calcium. The more accepted view is that it is a result of faulty calcium and phosphorus metabolism. Guassardo [25] suggested that this is due to an abnormal neuro-endocrine innervation. In 1931 Péhu, Policard, and Dufourt [26] found an adenoma of a parathyroid gland in one case of marble bones. Subsequently, generalized osteosclerosis was produced in rats with injections of parathormone. However, no corroborative experimental findings have since been published. Sutro [27] was able to produce osteosclerosis in mice with subcutaneous injections of estrogenic hormone. Pfeiffer, Kirschbaum, and Gardner [28] caused skeletal hyperossification in mice, pigeons, and sparrows with similar injections. The degree of hyperossification depended on the dose and length of administration of the estrogens. Howard and Gonzalez [29] reviewed the literature in reference to the chemico-physiologic aspects of osteopetrosis and found that no apparent intrinsic chemical reaction is responsible for the disease.

Schulze [30] conjectured that osteopetrosis is a healing process of osteomalacia or rickets. This appeared to be substantiated by Kudrjawtzewa [12] who found that the development of Albers-Schönberg disease is preceded by an osteoporotic process. The only other corroborative evidence is the occasional occurrence of rickets in cases of osteopetrosis.

Generalized osteosclerosis has been seen in patients suffering from phosphorus poisoning. These cases also simulate osteopetrosis in some respects, namely, in the necrosis and osteomyelitis of the mandible, disturbance of dentition, and even stratification of the long bones.

More recently, chronic fluorine poisoning has been considered an etiologic factor in marble bones. In 1936 Spéder [31] observed that many animals and human beings living in the phosphatic zones of Northern Africa developed generalized osteosclerosis. This was found to be due to fluorine which was included as calcium fluoride with the phosphorus. He noted similarities in the radiologic and clinical findings in marble bones and fluorine intoxication.

Anatomic studies of marble bones have been rare and incomplete. In 1934 McCune and Bradley [15] found that only 8 completely autopsied cases had been reported. In 1937 Gerstel [32] observed that of 100 cases cited by various authors, only 12 were studied from a pathologic-anatomic viewpoint, and in most of these no particular attention was paid to histopathology.

An analysis of the morphologic studies of marble bones indicates that certain alterations of the skeleton are constant, while others vary

with the degree of development of the disease and the age of the patient. These variations have led to different interpretations of the cellular structure, especially as it relates to the etiology and pathogenesis of the disease. Further histologic study is necessary in order to evaluate the various findings. According to McCune and Bradley,[15] Schmidt,[33] and others, the clinical and morphologic picture seen in infantile and juvenile cases is so different from the adult type as to suggest diseases of different pathogenesis.

Report of Case

A. E., a white female, was under the observation of the Department of Pediatrics of the Jewish Hospital from birth until death at 18 months. The infant was delivered spontaneously, appeared normal at birth, and weighed 7 lbs., 10 oz. The mother and father were first cousins. Their first child had died at 6 months of unknown causes; one sibling of 7 years was alive and well.

The patient was breast fed and progress was satisfactory until she developed an upper respiratory infection at the age of 4 weeks. At this time a tendency towards nasal bleeding was noted and the liver was palpated two fingersbreadth below the costal margin. The patient recovered uneventfully in 2 weeks.

At 10 weeks of age she was admitted to the hospital because of progressive anorexia, pallor, nasal discharge, and the development of frontal and parietal bosses. Examination revealed a well developed, well nourished, pale infant with a lusty cry. The motion of the extremities was active and unhindered. The temperature was 101°F., the pulse 96, and the respirations 28 per minute. There were prominent changes in the external configuration of the skeleton. Frontal and parietal bosses were seen and craniotabetic areas were felt along the sutures and behind the mastoid regions. The lambdoid sutures were separated 1 cm. The anterior fontanelle measured 3 by 3 cm. and was slightly depressed. The costochondral junctions of the ribs were thickened, angulated, and prominent. The wrists and ankles were widened. The pupils failed to react to light; the right was larger than the left. Funduscopic study revealed a "red retina" with pigmentation and atrophy of the optic nerves. The spleen was palpated 6 cm. below the left costal margin and the liver 4 cm. below the right margin. There was a generalized adenopathy and a number of petechiae were seen on the abdomen. A purulent nasal discharge was present.

The laboratory findings were as follows: Blood chlorides, 378 mg. per 100 cc. of serum; nonprotein nitrogen, 23 mg. per 100 cc. of serum; total protein, 5.2 gm. per 100 cc. of serum; cholesterol, 125 mg. per cent; sugar, 69 mg. per cent; calcium, 8.5 mg.; phosphorus, 2.4 mg.; calcium × phosphorus, 20; phosphatase, 10.8 Bodansky units; potassium, 19.6 mg.; albumin-globulin ratio, 1.1.

Studies of the peripheral blood showed the following: Hemoglobin, 45 per cent (6.5 gm.); red blood cells, 1,900,000 per cmm.; color index, 1.1; white blood cells, 13,900 per cmm. The differential count revealed 28 per cent polymorphonuclear leukocytes, 2 per cent staff forms, 26 per cent neutrophilic myelocytes, 12 per cent myeloblasts, 2 per cent metamyelocytes, 22 per cent lymphocytes, 8 per cent monocytes. Red blood cell study showed 60 normoblasts per 100 cells; macrocytosis, 4 plus; microcytosis, 2 plus; poikilocytosis, 2 plus; anisocytosis, 2 plus. A bone marrow smear contained very few cells, identified as mitotic red blood cells, myelocytes, myeloblasts, and erythrocytes. It was questioned whether the marrow cavity had been entered.

The urine was negative. Roentgenographic examination of the entire skeleton revealed a general increase in density of all of the bones.

In view of the blood calcium-phosphorus products of 20-32 and the elevated

phosphatase (9-13 Bodansky units), an accompanying rickets was suspected and the patient was treated with repeated small blood transfusions, large doses of viosterol and cevitamic acid. After 3 months of treatment the calcium-phosphorus product rose to 40-50 and the phosphatase decreased. The rickets was considered healed and the blood values subsequently remained at a normal level.

At the age of 4 months the patient had bronchopneumonia. At that time the lower border of the spleen was palpated at the level of the anterior superior iliac spine. The lymph nodes were all enlarged and soft. At 10 months the patient was able to hold up her head but she was unable to sit up without support. Two upper incisor teeth were present and one lower. She was completely blind. At this time she again developed bronchopneumonia from which she recovered in a few weeks. A general muscular atonia was then noted. At 14 months blood and marrow studies continued to show moderate leukogenesis and erythrogenesis, and deficiency in red blood cell maturation. At 16 months the fontanelles were closed, and external rotation and eversion of the lower extremities was noted. The chest appeared to be flattened antero-posteriorly. Severe bleeding from the mouth and nose, which occurred at this time, subsided spontaneously. A similar episode occurred at 18 months and the patient died of uncontrollable hemorrhage.

Roentgenographic examinations of the entire skeleton were repeated at monthly intervals until the time of death. The first, at 3 weeks of age, showed increased density of the bones of the entire skeleton, slight periosteal thickening, and roughening of the metaphyses of the long bones. The skull showed a similar increase in density, especially at the base (Fig. 1). There was wide separation of the bones at the suture lines.

Repeated studies of the skeleton in the ensuing months showed progressively increasing condensation. At 6 months the metaphyses and the epiphyses were developing normally. The long bones were wider than normal and of irregular contour. The cortex of the bones appeared to be less sclerotic than the remainder. Radiolucent striae were seen in the long bones, vertebrae, ribs, and pelvis. Some clubbing of the distal ends of the radius and ulna and of the proximal ends of the tibia was seen (Fig. 2). The ribs also showed increased density; the cortex, like that of the long bones, appeared broad, irregular, and less dense than the medulla. In places the entire width of the rib appeared to have the consistency of the less opaque cortical bone. Just distal to the tubercles of each of the first 10 ribs on both sides, there were single radiolucent striae. The most lateral portions of the bodies of the upper 8 ribs, bilaterally, showed sharp angulation (Fig. 3).

REPORT OF NECROPSY

The body was that of a poorly nourished, white, female infant, 66 cm. long and weighing 9 kg. The skin was pallid; there were numerous red areas around the umbilicus measuring up to 2 cm. in diameter.

The thyroid and parathyroid glands were not unusual.

The trachea and bronchi were filled with frothy fluid. The right lung weighed 70 gm.; the left, 60 gm. Preparations of both lungs showed small areas of extravasated blood in the pleura. The blood vessels were congested. Scattered collections of immature red and white blood cells were seen.

The gastrointestinal tract, liver, and kidneys showed no gross changes. On microscopic examination the sinusoids of the liver and the interstitial capillaries of the kidneys were distended with immature blood cells.

The spleen was firm; it measured 17.5 by 11 by 5 cm. and weighed 460 gm. On the cut surface, the malpighian corpuscles and the fibrous markings were indistinct; the pulp could not be scraped easily. Most of the sinuses were empty; some of them contained immature blood cells.

The lymph nodes were large and firm. The cut surfaces were homogeneous brick red. On microscopic examination the follicles were few and small. The pulp was very cellular. The sinuses were filled with immature blood cells.

On gross examination, the bones were heavier than is normal; they were hard and brittle. The cartilaginous portions were blue-gray and translucent; they cut easily.

The right tibia was slightly club-shaped at both extremities. The epiphyseal line was sharply demarcated and wavy; the zone of provisional calcification was a thin, faintly blue line. The cortical portion of the tibia consisted of dense, homogeneous, gray-white bone which appeared to merge with the compact medullary bone. Beneath the periosteum were a number of red patches.

A number of dorsal and lumbar vertebrae were removed and studied radiographically. The bodies consisted of dense bone. Central and peripheral concentric bands of rarer bone alternated with the more opaque bone. There were also seen oblique radiolucent striae which radiated from the center towards the periphery of the vertebral bodies. The cut surfaces of the vertebrae showed ill defined lines of separation between the cortex and the medulla. In the center of the bodies there was an oval, dark gray area of bone. A similar band of gray bone encircled the vertebra in the region midway between the cortex and the midcentrum. The gray areas in both the tibia and the vertebrae corresponded to radiolucent bands which were noted in the roentgenologic study.

Roentgenograms were taken also of the ribs after removal. The angular deformities previously noted were apparently due to fractures situated about 2.5 cm. lateral to the costochondral junctions. The distal fragments were displaced outward and the angulation measured up to 75°. The lateral portions of the 9th, 10th, and 11th ribs on both sides were also the seat of healed fractures, but there was no angulation. The callus at the sites of all of the fractures consisted of less densified bone of the same radiographic consistency as the cortical bone. On gross examination of the ribs a few small subperiosteal hemorrhages of the pleural superficies were found. The cut surfaces were compact, glistening white, and homogeneous. The sites of angulation consisted of overlapping, wedge-shaped ends of bone incorporated in gray bone callus.

Sections were taken from a number of different areas of each of the following bones: tibia, fibula, dorsal and lumbar vertebrae, and ribs. They were fixed in 10 per cent formalin and in Helly's solution, decalcified in 5 per cent nitric acid, and embedded in both paraffin and celloidin. Hematoxylin and eosin, May-Grünwald's and van Gieson's elastica stains were used.

Histologic Examination of Bones

Tibia

The germinal and proliferating layers of the epiphyseal cartilage of the tibia contained sparsely distributed fusiform and oval chondrocytes. Their cytoplasm was vacuolated and the nuclei were pyknotic. The zone of maturation was narrower than is normal; it consisted of irregularly placed chondrocytes of varying size and shape which lay singly and in isogenous groups in the hyaline matrix. The adjacent cells were aligned in short, irregular columns; at this point they were large, the cytoplasm was foamy, and the nuclei were karyolytic or pyknotic. Between the cells were narrow parallel columns of dense, blue-staining, calcified cartilage matrix. At the irregular medullochondral junction, the epiphyseal cartilage ended sharply. Adjacent to it there was seen in the juxta-epiphyseal portion of the metaphysis an irregular network of short, partly calcified cartilaginous trabeculae. These varied in shape but were of fairly uniform size and arrangement. The trabeculae consisted of an acellular, homogeneous, pale blue, lavender-staining substance, which was often mottled and granular. Occasional swollen chondrocytes appeared within this matrix; they were often encircled and at times completely covered by dense, blue-staining, amorphous calcium.

At the irregular chondro-osseous junction, the primary marrow cavities were small, isolated, and irregular. The marrow consisted of sparse, delicate connective tissue, occasional hematopoietic elements, and rare capillaries. Many of the medullary spaces were empty. The irregular dense blue borders of the cartilaginous trabeculae which lined the marrow cavities represented the fused edges of the original chondrocytic capsules. Their formation is known to follow the invasion of the degenerating cartilage cell columns by the primordial marrow. Upon the surfaces of many of the calcified cartilaginous trabeculae were deposited crescentic layers of dark-pink and pinkish blue-staining osteoid tissue. Many of the marrow cavities were filled with this tissue which showed evidence of having been deposited in concentric rings about capillaries as well as in excentric layers on the surface of the cartilage. The osteoid tissue appeared to form from the delicate fibrocellular marrow. Occasionally the connective tissue cells were seen

within the homogeneous pink substance and gave the appearance of branched osteocytes; in most cases, however, the osteoid tissue was acellular. In some areas, flat osteoblasts lined the surfaces of osteoid islands and produced appositional pre-osseous tissue. More rarely, chrondrocytes within the cartilaginous trabeculae were surrounded by osteoid rings and the cells resembled osteocytes (Fig. 4).

The shaft of the tibia consisted of a central compact network of calcified chondro-osteoid tissue which was enveloped by less dense cortical bone. Nowhere in the shaft was there a definitive bone marrow cavity. The bone was in a quiescent phase and showed no active alteration. Towards the midshaft the medullary bone was dense; the marrow cavities were partially or completely filled with osteoid tissue. The remaining minute myeloid areas contained either delicate connective tissue or were empty.

In the metaphysis of the tibia there was seen an irregular transverse band of bone which differed in appearance from the remainder of the shaft. It consisted of a less dense network of large bony trabeculae and wide marrow spaces. The trabeculae were composed of mixed primitive reticular bone, chondro-osteoid tissue, and early lamellar bone. On their surfaces there was moderately active osteoclastic resorption and osteoblastic deposition of new bone. The myeloid spaces contained both connective tissue and vascular hematopoietic marrow. In places the connective tissue marrow was loose and edematous and in its interstices were seen acidophilic bars of pre-osteal tissue. These portions of the metaphyses corresponded to the transverse bands of diminished density previously noted in the roentgenograms of the skeleton.

At another point in the tibial diaphysis the chondro-osteoid bone was replaced by vascular fibrocellular connective tissue, which was the seat of hemorrhage. Within this tissue were fragments of necrotic bone, many fibroblasts, lymphocytes, plasma cells, histiocytes, brown granular pigment, and a number of foreign body giant cells. Adjacent to this area there was active osteoclastic resorption of the chondro-osteoid tissue with widening of the myeloid spaces. Newly formed primitive bone was undergoing active reconstruction into compact lamellar bone (Fig. 5). This site apparently represented an incomplete fracture which, because of its small size, was not visualized in the roentgenograms of the tibia.

The cortex of the tibia was of varied thickness and presented a variegated appearance. Near the epiphysis it consisted of a single continuous trabecula of reticular bone which lay parallel to the fibrous, avascular periosteum. In the remainder of the shaft it was

composed of parallel and intercommunicating trabeculae which occupied increasingly wider areas from the epiphysis to the midshaft. In the latter situation the cortical bone occupied an area equal to one-third of the width of the entire tibial shaft. It was composed for the greatest part of trabeculae of mixed primitive and lamellar structure. Occasionally the reticular bone predominated and the trabeculae were covered with thin seams of lamellar bone. The large marrow spaces contained varied proportions of delicate connective tissue marrow and moderately vascular cellular marrow. Where the marrow appeared to be more vascular and the seat of hemorrhage, bone alteration appeared to be active. In places, numerous inactive osteoclasts were seen (Fig. 6).

The reticular trabeculae of the cortex were apparently replaced by lamellar bone from the center of the cortex, both inward and outward. This could be deduced from the prominence of lamellar bone in the midportion of this layer, while the outer, subperiosteal trabeculae and the inner endosteal trabeculae appeared to retain their primitive structure in most places.

In isolated areas the cortex consisted of dense, compact, lamellar bone. Numerous lines of growth on the periosteal surface indicated past periodic growth of the bone outwards, while the interrupted trabeculae incorporated in the substance of the compact bone, as well as the partially sclerosed marrow spaces, indicated a previous period of active endosteal bone deposition or inward growth. There was densely cellular fat-free marrow in the medullary spaces.

Vertebrae

Preparations from the bodies of the lumbar and dorsal vertebrae consisted of narrow outer zones of hyaline cartilage which enveloped a dense network of small, irregular, calcified, chondro-osteoid trabeculae. The minute marrow spaces contained delicate fibrous connective tissue. The general structure and appearance of this bone and the process of endochondral ossification resembled that described in the tibia. Within the body of each vertebra were two narrow concentric rings of bone which were analogous in structure to the metaphyseal radiolucent band previously described in the tibia. The outer ring consisted of large interconnected trabeculae of lamellar bone, some of which contained irregular masses of hyaline cartilage. These trabeculae were continuous with the adjacent denser chondro-osteoid tissue or they were separated from the latter by broad medullary spaces (Fig. 7). The marrow spaces contained cellular marrow, large blood vessels, occasional fat cells, and a slight amount of delicate connective tissue.

The inner central ring which was roentgenologically less dense consisted of an uninterrupted band of dense, cortical, lamellar bone which enclosed a large, central, marrow cavity and smaller peripheral spaces containing vascular, cellular marrow. Within the lamellar trabeculae were seen small remnants of reticular bone. A thin seam of primitive bone also lined the peripheries of this lamellar bone ring. Mature bone had replaced the reticular bone which appeared to have occupied this area previously; this process occurred from the center of this zone outward toward the periphery. Large, thick-walled blood vessels ran in straight lines from the perichondrium to the center of the bodies of the vertebrae; they were separated from the chondro-osteoid substance by parallel trabeculae of reticular bone. In their course through the vertebrae the blood vessels appeared to vascularize only the concentric bands of lamellar bone; no branches appeared to enter the chondro-osteoid zones.

Ribs

Preparations from the cartilages, epiphyses, and shafts of the ribs showed morphologic changes similar to those described above. The calcified chondro-osteoid trabeculae which formed the central core of the shaft were of the same architecture but they tended to lie parallel to the surfaces of the ribs. For the most part, the bone was in a quiescent phase. Only in portions of the central chondro-osteoid core where healing, incomplete fractures were seen were there active resorption and reconstruction of bone. The structure in these areas was similar to that previously described at the sites of partial fracture of the tibia. In some portions of the shafts the central chondro-osteoid core was replaced by larger trabeculae of mixed primitive reticular and lamellar bone of the same architecture and distribution as the bone which formed the cortex. This apparently represented complete healing of fractures and it was also found cementing the angulated ends of the fractured ribs. Occasionally, the entire width of the shaft was occupied by large interconnecting trabeculae which were made up of mixed lamellar and chondro-osteoid bone. The irregular distribution of these elements in the trabeculae gave them a mosaic appearance. The large medullary spaces in these areas contained vascular cellular marrow (Fig. 8).

The periosteum of the ribs consisted of a dense hyalinized fibroelastic outer layer and an inner congested fibrocellular layer; in places it was thickened. Hemorrhagic extravasations were seen between the inner layer of the periosteum and the cortex of the rib. At these sites in the cortex, membranous new bone formation followed on the heels of rapid bone resorption. The structure of the cortex was essentially

similar to that of the tibial cortex. It differed only in that the bone was more sclerotic, compact, and quiescent. In some areas it assumed the pattern of the normal skull; it then consisted of an outer compact table of bone, a central spongy layer, and an inner compact table.

Bone Marrow

It was noted that the myeloid cavities of all of the bones were occupied by different varieties of marrow, depending largely upon the type of bone which surrounded them. For the most part, where the trabeculae were made up of imperfectly composed chondro-osteoid bone the spaces were empty or they contained delicate fibrous connective tissue marrow with a minimal scattering of hematopoietic cells. In the myeloid spaces surrounded by relatively mature lamellar bone the marrow presented a fairly normal appearance. The adipose tissue was easily identified but it was relatively diminished and irregularly distributed. Blood cells in different degrees of maturation, from primordial hematocytes to mature forms, were abundant. Well developed blood vessels, chiefly arterioles with thickened walls, could be seen also in these areas. The spaces, which were bounded on one side by lamellar trabeculae and on the other side by chondro-osteoid tissue, contained a mixture of loosely arranged fibrous tissue cells with oval or vesicular nuclei, a few fat cells, and cellular marrow. Though many erythrocytes and leukocytes were found here, relatively few immature blood cells could be recognized. In some sites, hemorrhage, cell disintegration, and fibrous connective tissue replacement were seen. In some portions of the cortex where reticular bone predominated, mixtures of fibrous and cellular marrow were present; only occasional areas of cellular bone marrow were seen.

Discussion

Histomorphologic study of the skeleton in this case revealed a number of unusual structural changes in the epiphyses, metaphyses, and diaphyses of the long bones and in the bodies of the short bones not heretofore fully described in the literature.

In the preparations of the epiphyses and metaphyses some of the conditions which are necessary for normal growth of bone were not present. The epiphyseal cartilage was inadequately prepared for its part in the mechanism of endochondral ossification and a normal, cellular, vascular, primary marrow was lacking. The different zones of cartilage cell proliferation, maturation, and degeneration were small and poorly developed. The zones of column formation were narrow and irregular; in places they consisted of but two or three cell layers. In

place of the usual vascular marrow at the epiphyses there was seen avascular fibrous connective tissue. This apparently lacked the capillary mesenchymal properties which are necessary for the adequate penetration and dissolution of the short cartilage cell columns, with the result that there was formed at the medullo-chondral junction a scaffolding of short irregular bars of calcified cartilage matrix and minute marrow spaces. Instead of osteoblastic deposition of thin acidophilic osteoid seams on the surfaces of the cartilage spicules, there was seen here an overabundant deposition of calcified osteoid tissue both on the surface of the cartilage bars and in the marrow spaces between them. This tissue appeared to be formed largely by direct fibro-osseous metaplasia of the fibrous connective tissue marrow; less often, it was formed through osteoblastic deposition or through chondro-osseous metaplasia. Instead of the normal, delicate scaffolding of chondro-osteoid spicules and large myeloid spaces containing myelopoietic marrow, there was formed in the metaphyses of this case a dense network of chondro-osteoid tissue and minute marrow spaces containing connective tissue marrow.

Perversion of ossification in the submetaphysis in this case appeared to be due to the absence of osteoclastic and chondroclastic marrow cells. These are essential in resorbing the metaphyseal chondro-osteoid trabeculae in preparation for their replacement by more mature secondary lamellar bone in the submetaphysis. Their absence prevents the alteration of the dense chondro-osteoid tissue so that it remains unchanged in the submetaphysis and no lamellar bone is produced. Mature bone can be formed only when the initial processes of intrachondral bone formation have been completed.

Aberrations from the normal were seen also in the diaphyses of the long bones. Under normal conditions of early bone growth the medullary portion of the shaft consists of mixed trabeculae of chondro-osteoid and lamellar bone and cellular marrow. In the later stages the spongy bone of the submetaphysis appears to merge into the cortex and the diaphyseal spongiosa is resorbed. As a result, the diaphysis of a normal bone ultimately consists of a compact cylindrical cortex and a central definitive marrow cavity. In this case the normal pattern of cancellous bone and of a central marrow cavity was not seen. Instead, it consisted of the hypo-ostotic, sclerotic, calcified chondro-osteoid substance which has been described.

Unusual structural changes were seen also in the periosteal and cortical portions of all bones. According to most of the literature on Albers-Schönberg disease, the cortex is said to be unaffected. In this case the cortex consisted predominantly of layers of parallel trabeculae

of reticular bone and less often of sclerotic lamellar bone. It was irregularly widened in many areas. Occasionally there was seen a concentric, endosteal increase in width without alteration of the outside diameter of the shaft. More often there was also excentric periosteal widening of the cortex with a resultant broadening of portions of the shaft. The cortex of the bones contained less vascular and hematopoietic tissue than is normally found. In places, however, it was vascular and the seat of hemorrhage. Pease, DeSanctis, and Alter [34] reported numerous subperiosteal hemorrhages in their cases. Gerstel [32] described "braune Herde" on the surfaces of almost all bones. These consisted of hemorrhagic areas where the bone was rapidly altering and giant cells were numerous. Similar areas were seen in this case. In still other portions of the cortex numerous osteoclasts were seen on the surfaces of the bone trabeculae but little or no bone resorption was present. There appears to be no anatomic explanation for the inhibited osteoclastic function of the cells in these areas.

When the shaft of a normal bone is once formed, all normal ossification except that at the epiphyseal line takes place in membrane. Membranous ossification is also the most common form of pathologic new bone formation. As a rule, this type of bony growth is not preceded by an intermediate stage of calcification. However, in a number of preparations of the bone in this case there was preliminary calcification of the hyalinized connective tissue of the periosteum and a subsequent alteration and osseous replacement of this calcified fibrous tissue. This type of fibro-osseous metaplasia resembles the process seen in heterotopic ossification which is always preceded by calcification.

The histologic nature of the radiolucent bands has not been commented upon heretofore in the literature. Pirie [5,18] thought that radiologically they resembled the condensed lines of growth that are caused by illness in the young, *i.e.*, the lines that appear following healed rickets, in lead and phosphorus poisoning, in hypothyroidism, and in scurvy. Herscher and Stein [35] conjectured that the alternate lines of varied density may represent alternate progress and recession of the disease. We have noted that these striae stand out in sharp anatomic contrast to the remainder of the bone; they consist of large trabeculae of predominantly mature lamellar bone and wide marrow spaces containing vascular cellular marrow. The relatively normal histologic appearance of these bands favors the concept that they represent periods of remission or attempts at healing. This theory does not, however, explain the fact that the striae are constantly found in the well vascularized regions of the bones which are portals of entry for the nutrient blood vessels. It appears that the bands of radiolucent, relatively

normal bone in osteopetrosis are merely an expression of the relatively normal vascularity of these areas.

It is noteworthy that the skeleton in Albers-Schönberg disease appears generally to be poorly vascularized. The cortex of the bones as well as the regions of healing or healed fractures and the radiolucent striae contain a relatively better blood supply; in these areas the structure of the bone and marrow approaches normal. It appears that diminished vascularity is a factor in the hyperostosis and hypermineralization which are found in marble bones. Leriche and Policard [36,37] and later Jones and Roberts [38] noted that the amount of calcification of tissue depends on the blood supply. It has since been universally accepted that hyperemia leads to decalcification, and bone resorption and anemia to calcification and new bone formation. The lack of normal blood supply in young bone produces no alteration of growth in length. Latarjet [39] ligated the nutrient artery of long bones without affecting their growth. Only in venous stasis, such as occurs in cases of congenital varices as reported by Bier,[40] does elongation of bone occur. Busch [41] showed that when the blood vessels of the fingers are occluded a widening of the long bones occurs; the cortex becomes sclerotic and the marrow cavities are replaced by new bone. The hyperostosis, hypercalcification, and widening of the bones in osteopetrosis may therefore be manifestations of impaired blood circulation.

Bone Fragility in Marble Bones

The osteopsathyrotic propensity in marble bone disease has often been commented upon. Best and Taylor [42] believed that it is due to the disproportion of mineral to organic substance. They stated that the hardness, strength, and rigidity of all bone is conditioned by the balance of organic (fibrous) and inorganic (mineral) constituents, much as the same properties of plaster bandages depend upon the impregnation of cotton mesh with plaster of Paris. The cotton bandage possesses tensile strength but no rigidity, while a plaster cast is rigid but brittle. A proportionate amount of both materials is necessary for strength and resiliency. In osteopetrosis the mineral content of the bones is increased and, as a result, the bones are brittle and easily fractured.

Kramer, Yuska, and Steiner [16] made a chemical analysis of the bones in a case of osteopetrosis to determine whether the nature of the mineral elements as well as their concentration was responsible for bone fragility. The skeleton was found to contain a tertiary calcium phosphate salt similar to that isolated from normal bones; only occasional epiphyseal portions of the diseased bone contained secondary calcium

phosphate salts, a molecular structure which is not found in the normal skeleton. A final conclusion cannot be drawn from the findings in one case, but it appears from their study that the chemical quality of the calcium salt does not play a dominant rôle in the mechanical deficiency of the bones. Further investigation of this problem is indicated.

The relationship of the organic constituents of bone to bone strength has not been dwelt upon in the literature. Examination of the preparations of the skeleton of this case indicated that this factor may play an important rôle. The organic component of normal bone constitutes 30 to 40 per cent of its substance; it consists of the matrix, which is composed of bundles of collagenous fibers arranged in the pattern of lamellae, cementing osseo-albuminous and mucinous material, and osteocytes. The arrangement of the collagenous fibrils into closely cemented plate-like lamellae which run in alternating longitudinal, circular, and oblique directions give this structure its effective maximal strength similar to that attained in the construction of plywood. The skeleton in Albers-Schönberg disease contains relatively little mature lamellar bone; it consists predominantly of chondro-osteoid tissue. The latter does not have the quality, quantity, or arrangement of collagenous fibers and cement substance which is found in normal bone; it is therefore structurally weak. The portions of the cortex which are formed as a result of metaplasia of periosteal connective tissue and of fibrous reticular bone appear to have the same deficiency of the matrix. It is axiomatic that the mechanical value of bone is inversely proportional to the histologic differentiation of the tissue from which it is derived. It may be that hypermineralization and widening of the bones in Albers-Schönberg disease is but a mechanism to compensate for the poor quality of the organic matrix.

In addition to the above factors, the strength and rigidity of bone are also dependent upon the structure and architectural arrangement of the trabeculae which form it. Normal bone trabeculae are shaped in the form of tubes, plates, globes, and cylinders, each of which is fitted for a definite mechanical function. Furthermore, these trabeculae are arranged purposefully in the direction of the lines of maximum pressure or tension acting on a particular bone. The trabeculae of the bones in Albers-Schönberg disease follow no particular shape or pattern and their arrangement does not appear to have been influenced by the trajectories of stress and strain.

Pathogenesis

Controversial opinions have been offered on the pathogenesis of marble bones. Some investigators have contended that it is primarily a

disease of the osseous system and that the bone marrow and blood vessels are affected only secondarily (van Creveld and Heybroek,[23] Lorey and Reye,[24] Bernhardt,[43] Kopylow and Runowa,[44] and others). They have maintained that the mechanical encroachment of the bone upon the marrow produces myelophthisic anemia. To substantiate this opinion they have observed that there is no apparent relationship between the degree of bone alteration and the degree of marrow change and anemia. Many cases with severe osteosclerosis show little or no anemia (Clairmont and Schinz,[45] Howard and Gonzalez [29]) while others with minimal osseous change have marked anemia. Pease, DeSanctis, and Alter [34] also expressed the belief that osteopetrosis is a disease of osteogenic origin. In support of this idea was the observation that no normal bone was seen in the case reported by them. They reasoned that this differentiated their case from those of osteosclerosis secondary to diseases of the bone marrow, since some normal bone would be found in the latter. Other authors have suggested that marble bones is a disease of the blood-forming organs. Assmann [2] considered it to be some odd form of anemia or leukemia. Klemperer [46] was also of the opinion that it is a dyscrasia of the marrow. He postulated that young bone marrow has potentialities of forming both connective tissue and bone, and, furthermore, that the type of tissue which differentiates from it depends upon the stimulus. He cited the fact that under the influence of x-ray exposure bone marrow shows fibroblastic proclivities and little or no hematopoietic tendency.

It has been conjectured by some authors (Kudrjawtzewa,[12] Hässler and Krauspe,[13] Clifton and Frank,[47], Clifton, Frank, and Freeman,[48] and Grasser [49]) that marble bones is a disease of the undifferentiated mesenchymal anlage of the skeleton and bone marrow. Ontogenetically, the primordial mesenchyme is the common progenitor of both the hematopoietic and osseous systems. From the sclerogenous portion is produced the membranous anlage of the skeletal system which in turn is differentiated into the cartilaginous skeleton. From the myelogenous portion of the mesenchyme is differentiated the vascular bone marrow which assumes an important rôle in the differentiation of the cartilaginous skeleton into the definitive osseous skeleton. The latter process takes place in the first months of development of the bones when the blood vessels of the perichondrium accompanied by undifferentiated myelogenous mesenchyme grow into the hyaline cartilage and forms the primitive marrow cavities.

The histomorphologic changes described in the bones in our case appear to support the theory that marble bones is a disease of the primitive osseomedullary anlage. The clinical and anatomic variations of the

disease are apparently contingent upon the degree of involvement of each of the elements upon whose integrated growth and development depends the structure of the skeleton. It would appear that when the sclerogenous elements are predominantly involved, the disease takes a slow, benign clinical course even though the structure of the bones is greatly altered. When the myelogenous mesenchyme is seriously affected, as is the case in many of the infantile forms of the disease, the course is malignant and rapidly fatal. Between these two extreme forms there exist the varied pictures of marble bones which have been described in the literature.

SUMMARY

Following a discussion of the salient anatomic and roentgenographic features of marble bones and a review of the incidence and etiology of this disease, an illustrative case is presented.

A detailed study of the histomorphology of representative bones of the skeleton revealed changes in the epiphysis, metaphysis, diaphysis, and cortex, which appear to confirm the concept that the pathogenesis of osteopetrosis is related to diseased vascular and osseomedullary anlage.

There is no apparent resemblance of the histologic structure of the bones in osteopetrosis to that in other bone conditions which have been associated in the literature with the pathogenesis of this disease, *i.e.*, rickets, osteomalacia, lues, nonspecific inflammatory disease, phosphorus and fluorine intoxication, and osteosclerosis produced by estrogenic hormones.

Bone fragility in marble bones is due to the disproportion of mineral to organic substance, the poor quality and arrangement of the organic elements of the bones, the uncontrolled variability in size and shape of the bone trabeculae, and their purposeless architectural arrangement.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 123

FIG. 1. Skull showing condensation of the bones, especially at the base. Suture lines are widely separated.

FIG. 2. Sclerosis of both tibiae and fibulae; somewhat less dense bone in the cortex and in the region of the transverse radiolucent striae. The epiphyses are normal. The ends of the tibiae are clubbed.

FIG. 3. Increased density of the ribs, vertebrae, and pelvis. In the ribs, there are radiolucent striae in the vertebral portions and radiopaque angulation and fractures in the lateral aspects of the bodies. In the ileum, radiolucent striae run parallel to the crest.

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Pines

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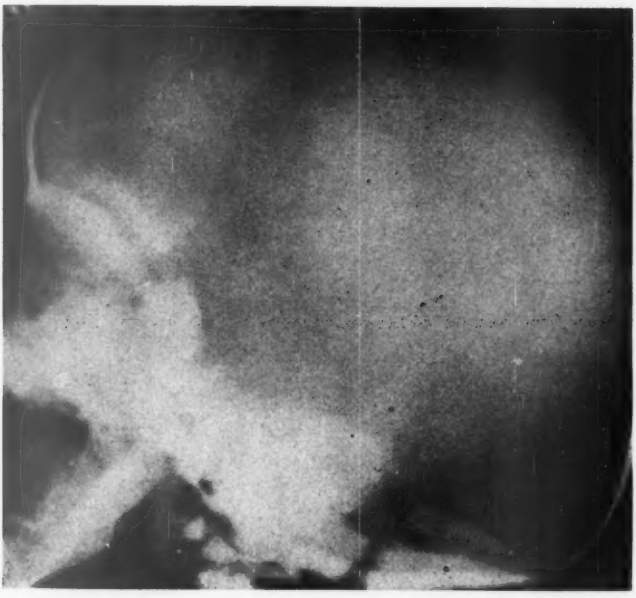

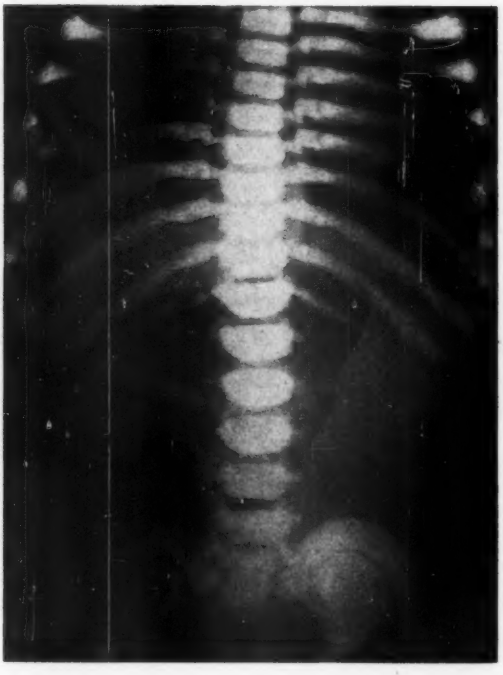

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Pines and Lederer Osteopetrosis

PLATE 124

FIG. 4. Section through the epiphyseal cartilage and metaphysis of a tibia. Hematoxylin and eosin stain. × 70.

FIG. 5. Section through the site of a healing fracture of the tibial diaphysis. Of note is the partial replacement of fibrocellular connective tissue by compact primitive and lamellar bone. Hematoxylin and eosin stain. × 65.

FIG. 6. Section through a portion of the cortex of a tibia consisting of parallel interconnecting trabeculae of primitive reticular bone. The marrow spaces are wide. Numerous inactive osteoclasts are seen. Hematoxylin and eosin stain. × 105.

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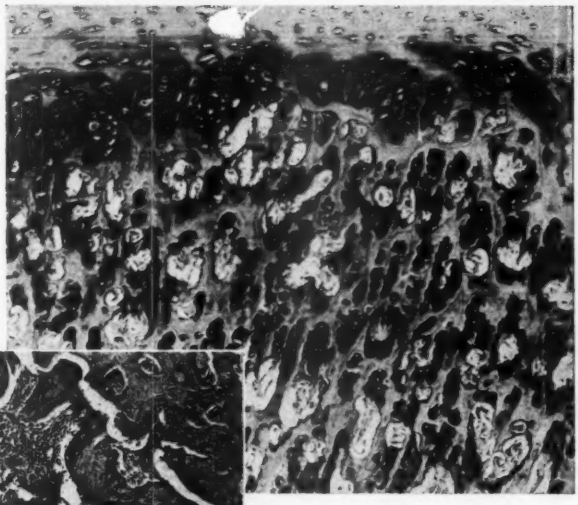

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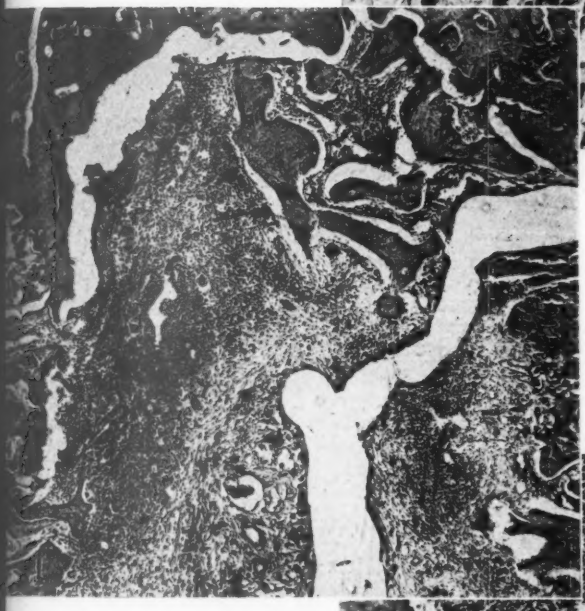

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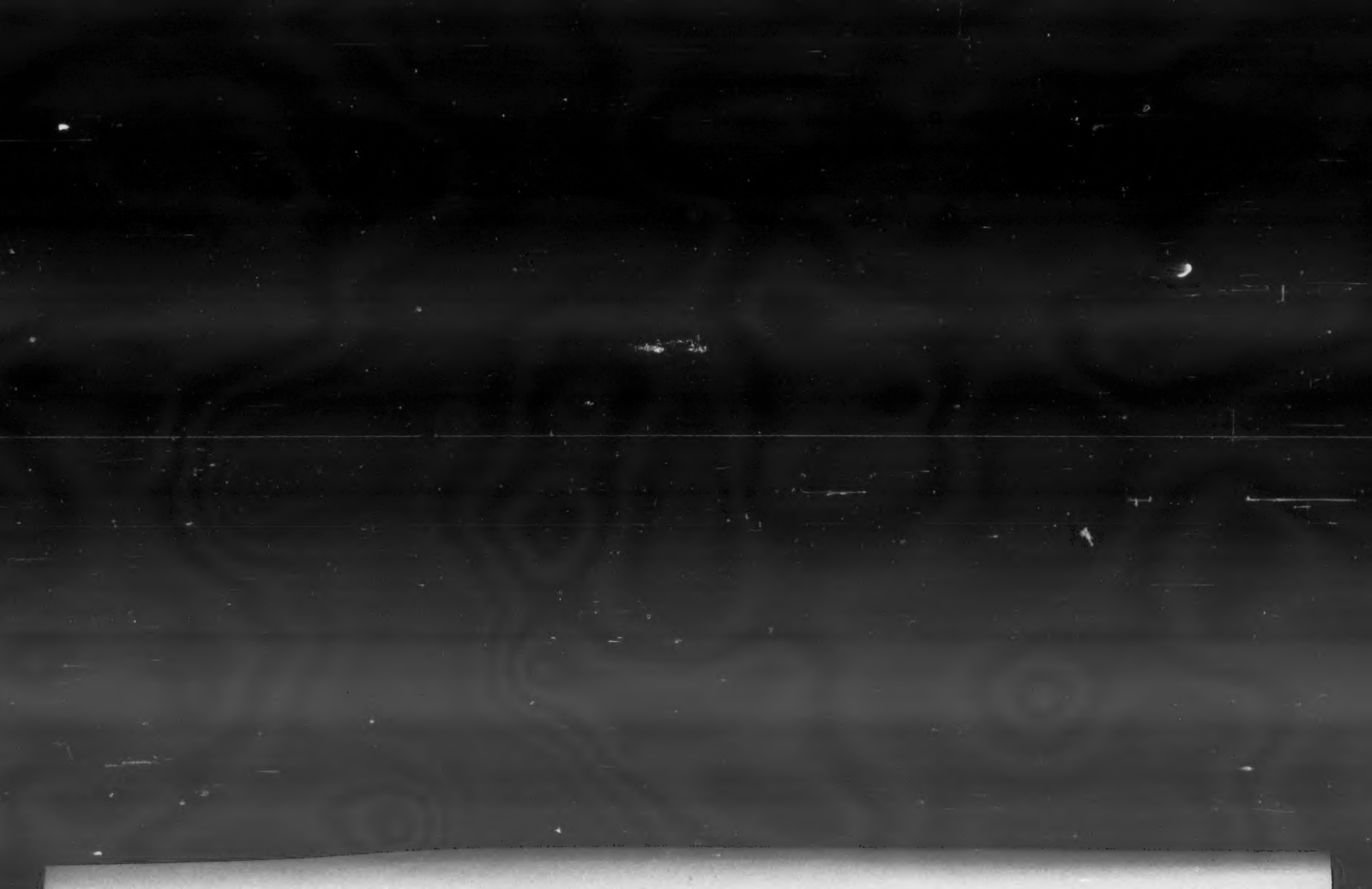
Plate 125

Fig. 7. Section through the body of a lumbar vertebra showing dense chondro-osteoid tissue on either side of a hypo-ostotic band of bone which consists of large lamellar bone trabeculae and marrow spaces filled with vascular, cellular marrow. The latter area corresponds to the radiolucent striae noted in the roentgenograms of the bones. Hematoxylin and eosin stain. × 130.

Fig. 8. Section through the body of a rib composed of mixed lamellar and chondro-osteoid elements. The trabeculae have a mosaic appearance. Hematoxylin and eosin stain. × 120.



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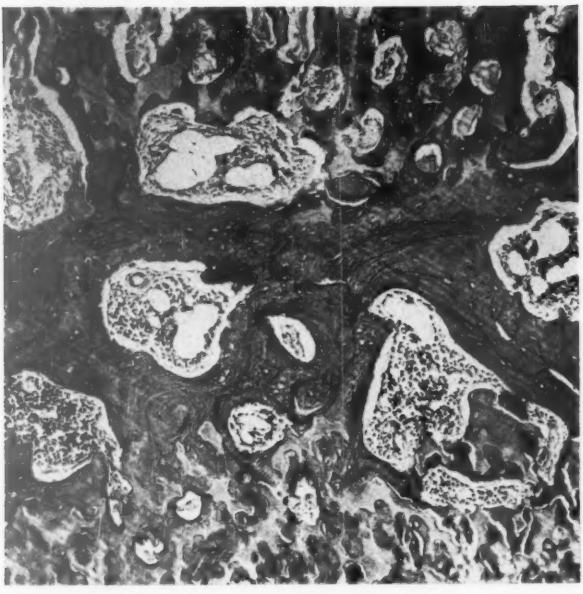

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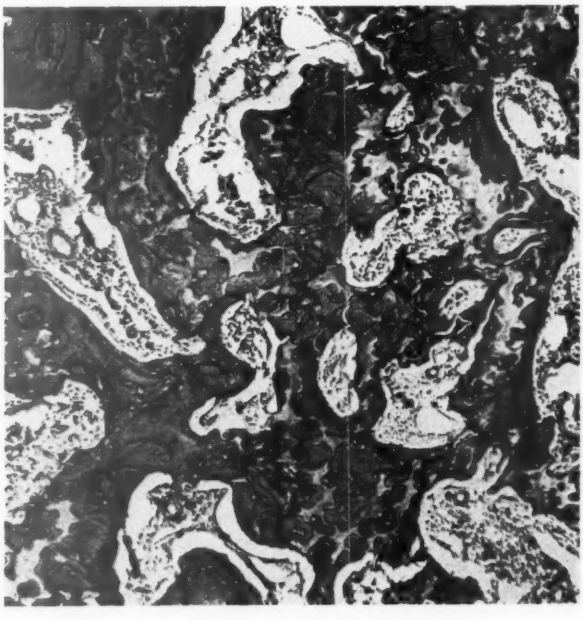

Pines and Lederer Osteopetrosis

EXPERIMENTAL ARGYROSIS

III. Pigmentation of the Eyes of Rats Following Ingestion of Silver During Long Periods of Time *

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The term argyrosis was introduced by Junge,[1] in 1859, to denote pigmentation of the conjunctiva and other parts of the eye by silver. More recently it has been used in a more general sense to include pigmentation by silver of the skin and various organs and tissues. In the course of an investigation of the effects of silver on the kidney and other organs, several hundred albino rats have been given solutions of silver salts instead of drinking water for varying periods of time that frequently approximated the normal life span of the animals. Their eyes became increasingly dark as this treatment continued. At autopsy, granules, apparently of silver or silver oxide, were deposited especially in the hyaline membrane of the choroid. It is the purpose of this communication (1) to indicate the ocular lesions of argyrosis found in man as reported by others; (2) to describe and correlate the ocular lesions found in the experimental animal during life and at autopsy, with especial reference to pigmentation in the hyaline membrane of the choroid; (3) to indicate points of significant difference between the site of deposition of silver in man and in the experimental animal.

Pigmentation of the human eye by silver usually follows local medication or occupational exposure to salts or organic preparations of silver, but may be part of generalized argyria. In local argyrosis, the conjunctiva and, to a lesser degree, the cornea are especially affected. In 20 human patients on whom otherwise reasonably complete autopsies were performed, the ocular lesions were studied only twice. Frommann [2] demonstrated silver, but his positive findings were limited to the bulbar conjunctiva. Küster [3] added a study of the eye to an autopsy reported by Riemer in 1875 and 1876. This concerned a 43-year-old man who had taken 34 gm. of silver nitrate internally. Küster described silver in finely granular form adjacent to the connective tissue strands and in the vessels of many parts of the eye. It was found in especially large amounts in the tunica propria of the conjunctiva, the subconjunctival tissue, the sclera, the dural sheath of the optic nerve, the capsule of Tenon, and the tendons and interstitial connective tissue of the extrinsic muscles. He could not demonstrate silver in the uveal tract, including the ciliary muscle, because of the

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deep natural pigmentation of these parts. However, he considered it present, in all probability, at least in the tunica choriocapillaris, the tunica vasculosa, and the interstitial connective tissue of the ciliary body. His reasons for believing that silver was present were in part based on analogy, but even more significant was the fact that he had demonstrated silver in the wall of a ciliary artery and postulated its presence in the entire area of distribution of this vessel. No silver was found in the parts not nourished directly by arteries, namely, the epithelium of the cornea and conjunctiva, the corneal substance, the lens and its capsule, the zonula, and the vitreous humor. Neither was it found in the optic and ciliary nerves, in the retina itself, nor in its vessels.

In a review of many clinical reports in which silver was recognized either by biopsy of the subepithelial tissue of the conjunctiva or by slit lamp or other devices in the cornea, I have found no other reference to pigmentation of the choroid or ciliary body in man by silver.

Study on experimental animals has usually demonstrated only superficial pigmentation by silver. For instance, silver has been applied to the surface of the eye by Gruber,[4] Dieter,[5] and Russo.[6] It has been injected under the conjunctiva by Weymann[7] and Fiore.[8] It has been injected intravenously by Gerlach.[9] By none of these methods was a generalized deposition of silver established. In 1873 Huet[10] reported feeding some rats, one for as long as 14 months, on silver nitrate powder mixed with sugar on bread. Dark pigmentation of the eyes was found during life but silver was not demonstrated on microscopic examination.

Experimental Methods and Results

The rats used were albinos of a strain originally coming from Rockland Farms, New City, N.Y., and most of them had been bred in the department for generations. They were given adequate amounts of dog chow, which has been found to be a complete diet for growth and reproduction. Instead of the water given the controls, the experimental animals were given solutions of silver salts. Of the 159 rats whose eyes were studied during life or at autopsy or, generally, in both ways, 143 received a 1:1000 solution of silver nitrate while 16 received a 1:1000 solution of silver chloride held in solution by about 3.5 times as much sodium thiosulfate as silver salt. Eighty-two of the animals were males, 55 were females who had had no litters, and 22 were females who had had at least one litter. The silver solutions were kept in dark bottles and usually given to the rats from about the time of weaning at about 1 month old until their death. In occasional rats, silver was given for a period, after which it was stopped and water sub-

stituted for the rest of the life of the animal. This procedure caused no diminution of pigmentation of the eyes. Following the ingestion of silver salts, there was no apparent change in the duration of life or incidence of infection. The weight of the treated rats was rarely below that of their controls. The incidence of pregnancy and parturition tended to be slightly diminished and lactation was often unsatisfactory, especially in females whose parents had received silver.

Observations During Life

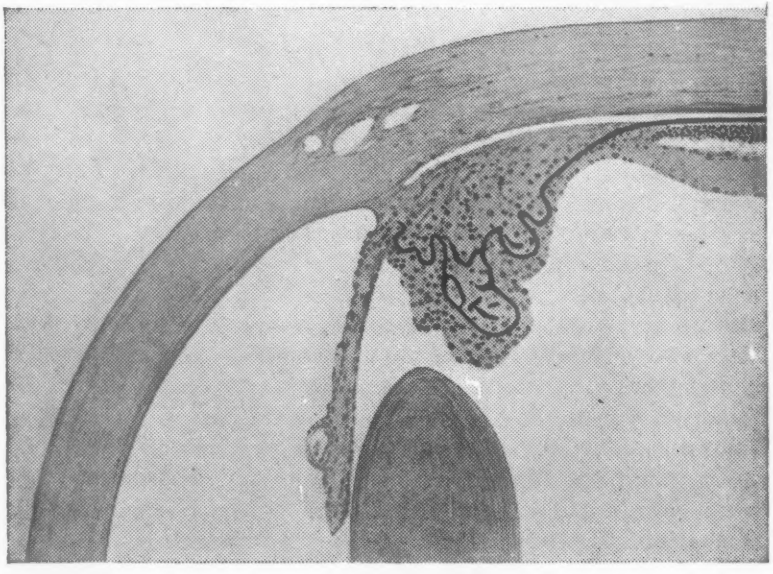
There was rarely any appreciable pigmentation of the skin of the silvered rats. The only significant difference observable during life between those animals receiving silver solutions and those receiving water was the pigmentation developing in the mouth and eyes of the former. The dark color of the eyes was in striking contrast to the bright red appearance of the eyes of untreated albino rats. The incidence of pigmentation was essentially the same in the animals given silver nitrate and in those given silver chloride with sodium thiosulfate. The incidence and degree of pigmentation was similar in males and females whether or not the latter had had litters. An attempt was made to determine objectively the degree of pigmentation of the eyes while the rats were still alive. The pigmentation of the eyes was described as follows: *1 plus,* when the eyes were slightly gray (found 98 times in animals which had received an average of 5.0 gm. of silver salt in an average of 218 days); *2 plus,* when the eyes were more gray than pink (found 84 times in animals which had received an average of 8.9 gm. of silver salt in an average of 373 days); *3 plus,* when the eyes were very dark but had preserved their translucence (found 37 times in rats which had received an average of 10.7 gm. of silver salt in an average of 447 days); *4 plus,* when they were even darker and appeared opaque (8 observations in animals receiving an average of 14.8 gm. of silver salts in an average of 553 days).

Autopsy Findings in Rats

For showing granules of silver in the eye and other organs, Bouin's solution is the most satisfactory fixative, although U.S.P. formaldehyde solution diluted with 9 parts of water can be used. Fixation with Zenker's or other mercuric solution is inapplicable as the necessary removal of the mercury with iodine and sodium thiosulfate [11] removes the silver also. Prepared sections of tissue taken at necropsy from a number of control rats given water and from 139 rats that had been given silver showed the following changes:

Conjunctiva. Silver has never been found in the epithelium of the

conjunctiva. It was regularly found as fine granules along the walls of vessels and in fine strands in the substantia propria of the conjunctiva between the conjunctival epithelium and the extra-ocular muscles at the fornix. As similar strands were stained clearly with Weigert's stain for elastic tissue in a rat receiving water, it is evident that the strands along which the granules of silver were deposited were, in all prob-



Text-Figure 1. Diagramatic drawing (by Mr. H. Murayama) of a portion of the eye of a rat based on the section shown in Figure 1. The dark line represents the choroid layer and its continuation as the basement membrane of the epithelium of the ciliary body. No silver is demonstrable in the iris or retina.

ability, strands of elastic tissue. No definite strands of elastic tissue were demonstrated in any other part of the eye of the experimental animals. Silver was regularly found to be laid down in small granules around the muscle strands of the extra-ocular muscles. It is not possible to determine whether these granules were deposited along the fine blood vessels or in the endomysium.

Cornea and Sclera. No deposition of silver has been found in the membranes of Bowman or Descemet, or in the corneal endothelium. In a few sections there were a very few fine granules in the substantia propria of the cornea. Also, in a few sections, fine granules of silver were deposited along the vessels and strands of scleral tissue. These

granules, though rare in both tissues, were slightly more abundant in the sclera than in the cornea. From a rat that had received water, an eye stained with Wilder's [12] silver stain showed slightly more pigmentation in Descemet's membrane than was found in the tissues in general. In our experience this technic is very valuable for various purposes but tends to be rather general in its staining so that this pigmentation cannot be considered specific.

Lens. No silver has been recognized either in the capsule or in the substantia propria of the lens.

Choroid. There were usually a few fine granules of silver around the arteries of the vascular layer of the choroid. More numerous and larger granules were found in the capillary layer. It is not clear whether this deposit was essentially pericapillary or whether it was laid down around the fibers on the scleral surface of Bruch's membrane, if such fibers exist in the rat. Certainly, no fibers stained characteristically for elastic tissue.

The site of constant and advanced deposition of silver was the homogeneous layer of the membrane of Bruch. Here the pigment varied from a light lemon color, often containing a few fine, dark granules, to an intense uniform black. In this region an attempt was made to correlate quantitative findings in the ocular sections with those found in other organs, as well as the clinical findings in the eye with the histologic picture. The silver can be removed readily by treating the sections with iodine followed by sodium thiosulfate as used for removing mercury after sublimate fixation.[11] The homogeneous layer of the membrane of Bruch, in an animal that has not received silver, stains like connective tissue with Masson's and Mallory's technic. It does not stain in a way characteristic of elastic tissue with Weigert's stain. When Wilder's [12] silver technic is used, this layer stains darker than any other ocular tissue. Even here, though, the staining is much more fibrillary and less homogeneous than in the rat that has ingested silver for long periods.

Ciliary Body. Pigmentation was regularly present in the membrane underlying the epithelium of the ciliary body. This is of the same magnitude as that found in the homogeneous layer of Bruch's membrane with which it is continuous. No pigment was found in the epithelium itself. The membrane of the control rat stained much less uniformly with Wilder's [12] technic than did those of the rats ingesting silver. No silver was demonstrated clearly in the deeper tissues of the ciliary body.

Iris. No pigmentation was present in the iris.

Retina. No pigmentation due to silver has been found in the layer described in man as the "pigmented layer of the retina." Since all of the rats were albinos, this layer was also devoid of ocular melanin and was entirely unpigmented. No silver has been found in the other layers of the retina.

Optic Nerve. Silver was deposited in moderate amounts in the outer layer of the optic nerve and of its vessels as they entered the eyeball.

Grading of Amount of Deposition of Silver

The amount of silver deposited, with particular reference to the homogeneous layer of the membrane of Bruch, was graded as follows:

One plus, when there were a few, but definite granules.

Two plus, when there was a moderate number of granules. The membrane was usually light yellow between them.

Three plus, when Bruch's membrane was dark brown and the granules formed an almost uninterrupted band.

Four plus, when the membrane was an almost uniform black.

There was good correlation between the pigmentation as observed during life and in histologic sections. Of 111 rats, 59 showed the same degree of pigmentation during life and at autopsy, while in 49 there was a variation of only one grade, with a variation of two grades in the remaining 3 animals. The relative grade of pigment deposition in the eyes of 139 rats was the same as in other organs, chiefly the kidneys, in 25; one grade less in the eyes in 93, and two grades less in the eyes in 21.

It will be seen that the finding in the eyes of rats agree substantially with those described in man by Küster.[3] However, the results in the choroid of the albino rat can be interpreted more precisely than in man because of the absence of any other confusing pigment. In man and the rat the deposition of pigment appears to be determined by the relative amount of vascularity in the various parts of the eye. Certainly, the maximal deposition of silver is present in the especially vascular choroid and ciliary body of the experimental animal. The almost complete lack of deposition of pigment in Descemet's membrane in the rat is in interesting contrast to the usual findings in man.

The localization of silver in the eye is in conformity with the selective localization in vascular areas in other tissues of the body. In material from our rats and from human autopsies, the chief extra-ocular sites where silver is laid down are: the basement membrane of the glomeruli and of the tubules of the kidney, the walls of the portal veins in the liver, the vessels of the thyroid gland, and the choroid membrane of the brain.

SUMMARY

Ocular pigmentation due to the deposition of silver can be recognized during life and at autopsy in rats ingesting silver salts for long periods of time. The amount of pigmentation is directly related to the duration and intensity of the treatment. The zone of greatest pigmentation in the eye is the homogeneous layer of Bruch's membrane of the choroid and its continuation as the basal membrane of the epithelium of the ciliary body.

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[*Illustrations follow*]

DESCRIPTION OF PLATE

PLATE 126

FIG. 1. Photomicrograph from an eye of a male rat, 16 months old, that received a total of 14.3 gm. of silver chloride in sodium thiosulfate in 444 days. Pigmentation of the choroid and basement membrane of the ciliary body was graded 3 plus.

FIG. 2. Photomicrograph from an eye of a female rat, 20 months old, that received a total of 12.2 gm. of silver chloride in sodium thiosulfate in 514 days. Injection of this rat with 2,3-dimercaptopropanol (BAL) caused no apparent diminution of pigmentation during life or at autopsy.[13] The clear layer between the nuclei of the retina and the choroid corresponds to the pigmented layer of the retina of man.

Olcott

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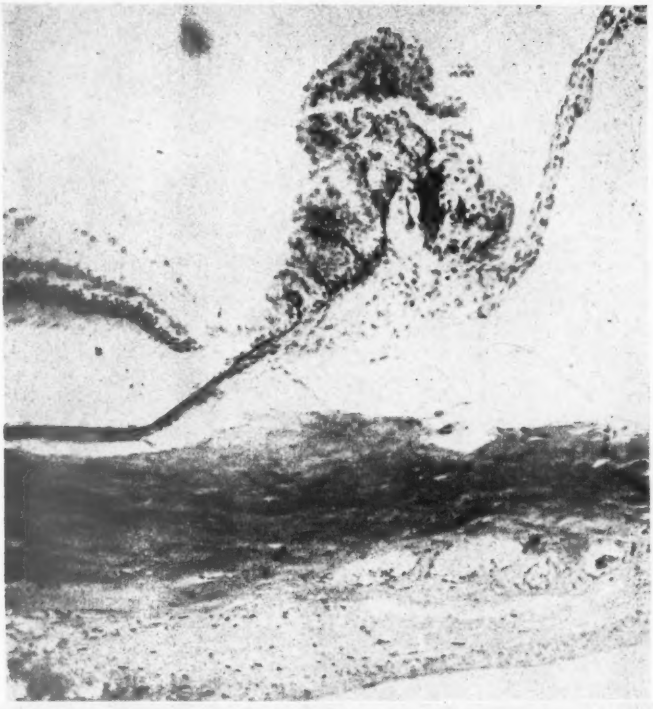

Olcott Experimental Argyrosis of the Eyes

THE RESORPTION OF ARTERIAL ATHEROMATOUS DEPOSITS IN WASTING DISEASE *

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A statistical analysis [1] has revealed that at necropsy severe atherosclerosis is at least twice as common in obese as in undernourished persons 35 years of age or older. This relationship proved to be independent of sex, hypertension, and diabetes. A large number of those found to be undernourished at necropsy had been well nourished or even obese prior to the onset of terminal wasting disease. If the degree of atherosclerosis that had developed in those cases was unchanged during the final illness, the relationship between atherosclerosis and overnutrition must be even more striking than was indicated; for if these individuals had developed marked atheromatous lesions during periods of average or overnutrition, their inclusion among the undernourished in a necropsy series would raise the incidence above its real value.

The alternative explanation is that during a period of marked weight loss, significant resorption of previously formed atheromatous deposits may occur. There is little evidence that such lesions in man are reversible. It is not unreasonable to suppose that early deposits consisting largely of lipids might be resorbed under suitable circumstances. Hyalinized or calcified intimal plaques may harbor resorbable material in their depths. Withdrawal of such deposits could lead to diminution in size of even advanced lesions.

The persistence of intimal deposits of cholesterol in the arteries of rabbits depends upon the continued feeding of cholesterol-rich diets (Krylov,[2] Anitschkow [3]). These deposits in rabbits closely simulate early human lesions. This suggests that the early human lesion is not necessarily permanent. Krisch,[4] Zinserling,[5] Aschoff,[6] and Ophüls [7] believed that the intimal lipid streaks noted frequently at necropsy in the aortas of infants and children may undergo spontaneous regression. However, because of the proximal location and diffuse character of such deposits, their relationship to adult atheromatosis has been disputed.[8] Leary [9] stated that cholesterol deposits in the atheromatous lesions of man may undergo lysis. The literature on this subject has been discussed in detail in the review by Hueper.[10]

The importance of determining whether or not atherosclerotic lesions are reversible at all is self-evident. If spontaneous resorption can

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occur, it is possible that the conditions leading to this event might be simulated. The present study was undertaken to determine if appreciable resorption of atheromatous lesions in the aorta and coronary arteries occurs in patients who lose considerable weight during a terminal illness.

Materials and Methods

Kodachrome photographic slides were prepared of the intimal surfaces of the aortas and main branches of the coronary arteries obtained at necropsy from 104 patients in Bellevue Hospital. Extensively calcified or narrowed portions of the coronary vessels were photographed in cross section. With a few exceptions photographs of both the aorta and the coronary arteries were available from each patient. In all, the state of nutrition during health, the duration of the final illness, and the amount of weight lost during that period were known.

The analysis was restricted to those from 40 to 60 years of age at death because it is usually at about 40 years of age that a significant degree of atherosclerosis becomes manifest, while at 60 years a high incidence is observed. Older patients were excluded because in them there might have been a long interval between the acquisition of some of the atherosclerotic plaques and death. Moreover, the state of nutrition of the individual may have been greatly altered in the interim.

The data concerning weight loss were supplied largely by the patients. Evidence of recent weight loss as noted on physical examination was used as a check. It is likely that some patients were unaware of, or denied, minor degrees of recent weight loss. Consequently, in the patients listed as not having lost weight, there may be some error. Striking quantitative differences in the amount of atherosclerosis observed in those listed as not having lost weight and those who had lost weight are, however, of significance for comparative purposes.

Thirty-nine of the group in which the coronary arteries were studied and 41 of the group in which the aortas were studied had lost from 15 to 110 lbs. in a period of from 1 to 12 months preceding death. The remainder in each group had lost less than 10 lbs. prior to death. Two in the coronary artery group and 3 in the aorta group had gained a small amount of weight terminally.

The evaluation of the state of nutrition at necropsy was based on the amount of adipose tissue deposited in the subcutaneous, mesenteric, omental, retroperitoneal, and perirenal regions. The standards were the same as those used in a previous analysis.[1]

The degree of atherosclerosis was measured by arranging the kodachrome slides, as unknowns, in sequence, using the extent of intimal surface covered by plaques of various types as the chief criterion.

Where the process was of approximately equal extent in any two, the one showing more advanced lesions, namely, those that were hyalinized, calcified or ulcerated, was considered to have the greater degree of involvement. The series was then divided into four groups of 26 cases each. The first group, with the least change, will be referred to as showing "minimal" lesions; the second group will be listed as "mild"; the third, as "moderate"; the fourth, as "severe." The sorting-out process was repeated three times and, although there were minor shifts in position within each group, in only isolated instances were these great enough to displace any individual case from one major group to another.

Results

The Relation of Terminal Weight Loss and Nutrition to Atherosclerosis

In Table I the number of cases of minimal, mild, moderate, and severe atherosclerosis of the coronary arteries found in the obese, average, and poorly nourished groups and the association with weight loss in each category are indicated. The relationship between nutrition and severity of atherosclerosis is striking. Twelve of the 24 obese, but

Table I

The Relation of Terminal Weight Loss and Nutrition to Coronary Atherosclerosis

Nutrition	Weight loss	Degree of atherosclerosis				Total
		Group I Minimal	Group II Mild	Group III Moderate	Group IV Severe	
Obese	With	0	0	0	3	3
	Without	2	3	7	9	21
Average	With	3	3	1	3	10
	Without	4	7	11	9	31
Poor	With	9	8	7	2	26
	Without	8	5	0	0	13
Totals	With	12	11	8	8	39
	Without	14	15	18	18	65

only 2 of the 39 poorly nourished persons had severe atherosclerosis of the coronary arteries. This relationship is even more pronounced than was reported in an earlier analysis [1] on a larger series of less well controlled cases. As in the earlier report, if the hypertensive cases are excluded, a relationship between severe atherosclerosis and overnutrition is still demonstrable. Both of the poorly nourished persons but only 6 of the 12 obese individuals with severe atherosclerosis had high blood pressures. Similarly, in this small series as in the larger one previously reported, the relationship between atherosclerosis and nutrition is independent of sex and age.

Among the 13 poorly nourished persons who had not lost weight terminally and who therefore were presumably poorly nourished for long periods of time, there were no examples of either severe or moderate atherosclerosis. All 9 of the poorly nourished persons who had moderate or severe atherosclerosis of the coronary arteries had lost weight prior to death and probably had been previously well nourished. It is thus seen that terminal weight loss tends to obscure the nutritional factor in the pathogenesis of atherosclerosis.

There were 5 obese persons in the series who had not lost weight, but who, nevertheless, had only mild or minimal atherosclerotic lesions. Therefore, obesity is not invariably associated with severe atherosclerosis. Furthermore, 2 of these 5 were not only obese but had hypertension as well. However, they were relatively young; one was a 45-year-old male and the other a 46-year-old female. The remaining 3 obese, nonhypertensive persons with little atherosclerosis of the coronary arteries were a 51-year-old male and 2 females, each 59 years old.

It may be noted also that 17 poorly nourished persons with weight loss prior to death had only minimal or mild atherosclerosis of the coronary arteries. When the degree of atherosclerosis was compared in all persons with or without terminal weight loss, a relatively large proportion of those with weight loss showed little atherosclerotic change. When the poorly nourished persons without terminal weight loss were excluded from this comparison on the grounds that they probably never had had significant atherosclerotic lesions, this relationship was strongly accentuated. Thus only 16 of 52 well nourished or obese persons (31 per cent) without terminal weight loss had minimal or mild atherosclerosis whereas 23 of 39 (59 per cent) with weight loss fell into similar categories.

The results of a similar analysis made on the aorta (Table II) are

TABLE II

The Relation of Terminal Weight Loss and Nutrition to Aortic Atherosclerosis

Nutrition	Weight loss	Degree of atherosclerosis				Total
		Group I Minimal	Group II Mild	Group III Moderate	Group IV Severe	
Obese	With	0	0	1	2	3
	Without	1	4	8	8	21
Average	With	4	3	2	0	9
	Without	4	8	8	10	30
Poor	With	10	8	5	6	29
	Without	7	3	2	0	12
Totals	With	14	11	8	8	41
	Without	12	15	18	18	63

almost identical, indicating that the relationship between atherosclerosis, state of nutrition, and terminal weight loss is not peculiar to any one artery. Of 29 poorly nourished persons who had lost considerable weight before death, 18 (62 per cent) had only minimal or mild atherosclerotic lesions in the aorta. Of 51 persons who were obese or of average weight and who had not lost weight prior to death, 17 (33 per cent) had only minimal or mild lesions.

There is thus suggestive evidence that less severe degrees of atherosclerosis in both the aorta and coronary arteries are observed in persons who had lost weight prior to death than in those who had not. The implication of this finding is that there may be significant regression of at least certain types of lesions in association with wasting disease.

The Relation of Macroscopically Visible Intimal Lipid Deposits to State of Nutrition and Wasting Disease

As previously noted, it is reasonable to expect that the lipid component of intimal lesions might undergo resorption more readily than hyaline or calcific material. For this reason, the kodachrome slides were rearranged in graded sequence according to the amount of visible lipid in the form of diffuse yellowish intimal deposits, as streaks, flecks, or circumscribed mounds. Each series was again subdivided into four equal groups of 26, designated consecutively as "minimal," "mild," "moderate," and "severe." In the rearrangement the presence of hyalinized, calcified, ulcerated, or hemorrhagic areas was disregarded. The new groups differed considerably from the original based on grading all types of atherosclerotic lesions.

In Table III the relation of the amount of grossly visible lipid in the

TABLE III

The Relation of Terminal Weight Loss and Nutrition to Lipid Deposits in Coronary Arteries

Nutrition	Weight loss	Degree of lipid deposition				Total
		Group I Minimal	Group II Mild	Group III Moderate	Group IV Severe	
Obese	With	0	0	2	1	3
	Without	1	3	6	11	21
Average	With	3	4	0	3	10
	Without	7	4	12	8	31
Poor	With	9	10	4	3	26
	Without	6	5	2	0	13
Totals	With	12	14	6	7	39
	Without	14	12	20	19	65

coronary arteries to state of nutrition and terminal weight loss is shown. Large amounts were found more often in the coronary arteries of persons who were obese or of average weight than in those who were poorly nourished. There were 20 obese persons and 23 of average nutritional state who had moderate or marked intimal lipid deposits, and in each of these groups only 3 had lost weight terminally. In the poorly nourished group only 2 without terminal weight loss showed moderate intimal lipid deposits and none had marked deposits.

Of the 26 poorly nourished persons who had lost weight terminally because of wasting disease, 19 had minimal or mild and only 7 more extensive deposits of intimal lipid. It may be inferred, therefore, that grossly appreciable depletion of lipid deposits usually occurs during a period of weight loss lasting several months.

TABLE IV

The Relation of Terminal Weight Loss and Nutrition to Lipid Deposits in Aortas

Nutrition	Weight loss	Degree of lipid deposition				Total
		Group I Minimal	Group II Mild	Group III Moderate	Group IV Severe	
Obese	With	0	1	0	2	3
	Without	2	3	7	9	21
Average	With	3	3	2	1	9
	Without	2	7	10	11	30
Poor	With	9	11	6	3	29
	Without	10	1	1	0	12
Totals	With	12	15	8	6	41
	Without	14	11	18	20	63

There were, however, 3 obese persons and 3 of average nutritional state as well as the 7 poorly nourished persons who, despite terminal weight loss, continued to show moderate or marked lipid deposits. The data available do not indicate that this group of 13 that failed to show reduction of intimal lipid had lost less weight or that the average period of weight loss was shorter or longer than in the group of 26 with terminal weight loss that apparently did have significant withdrawal of lipid deposits. Moreover, hypertension does not appear to be a factor in the failure of resorption of lipid. Only 4 of the 13 with moderate or marked amounts of persistent lipid had definitely elevated blood pressures.

Lipid deposits in the intima of the aorta bear approximately the same relation to state of nutrition and terminal weight loss as those observed in the coronary arteries. Relatively few obese or well nourished persons had only scanty lipid deposits in the aorta; relatively few poorly nourished ones had large amounts (Table IV). Of 41 persons, most of whom presumably had been well nourished or obese prior to

a terminal wasting disease, 27 had only minimal or mild deposits of lipid in the aortic intima. The remainder still had large intimal deposits.

It may be concluded, therefore, that during periods of weight loss, lipid deposits in the intima of arteries usually, but not invariably, tend to undergo resorption.

There are some differences in the distribution and character of lipid deposits in those who have lost weight terminally as opposed to those who have not. As a rule, the lipid deposits in well nourished or obese persons who have not lost weight tend to be sharply outlined or circumscribed, to project above the surrounding intimal surface, to be superficially located close to the endothelial surface, and to be fairly thick and opaque. Often in this group, the margins of older hyalinized plaques are surrounded by a narrow rim of yellow lipid.

On the other hand, in persons with wasting disease, lipid deposits are often thin and partly translucent as well as being scanty in amount. They tend to lie deep in the intima and to have frayed or indefinite borders. The intimal surface often is not raised in the areas of lipid deposit. Lipid deposits usually are not seen at the periphery of hyalinized plaques. However, these features are by no means constant.

The Relation of Hyaline and Calcific Intimal Plaques to Nutritional State and Terminal Weight Loss

There is little reason to believe that either hyaline or calcified plaques can undergo complete resolution or even partial regression except over long periods of time. Nevertheless, the series was analyzed to see if any appreciable difference in the number and size of such lesions could be observed in the arteries of those who had lost weight terminally as contrasted with those who had not. The kodachrome slides again were arranged in sequence according to the extent of hyaline and calcific plaque formation so that four new groups of 26 cases for each series were obtained.

It is apparent (Table V) that terminal weight loss had little effect on the extent of intimal involvement of the coronary arteries by hyalinized or calcified plaques. The cases with terminal weight loss were distributed almost evenly in the four groups. While there were 20 persons with terminal weight loss who had only minimal or mild formation of hyaline or calcific plaques, there were also 19 who had moderate or marked changes of this type. Analysis of the aortic series in the same manner revealed a similar negative correlation between weight loss and hyaline and calcific plaques. The data of this analysis are therefore not presented.

The results in Table V indicate, nevertheless, that a relation between

the general state of nutrition and the formation of these lesions does exist. Thus 13 of 21 obese persons without terminal weight loss had moderate or marked hyaline and calcific plaques. Only one of the 13 poorly nourished persons without terminal weight loss showed moderate hyaline or calcific plaques in the coronary arteries, and none had severe lesions. However, when terminal weight loss is ignored and the state of nutrition as observed at necropsy alone is considered, this relationship is obscured. Twelve of 39 poorly nourished persons, including both those with protracted undernutrition and those with terminal

TABLE V

The Relation of Terminal Weight Loss and Nutrition to Hyaline and Calcific Plaques in Coronary Arteries

Nutrition	Weight loss	Degree of hyaline and calcific plaque formation				Total
		Group I Minimal	Group II Mild	Group III Moderate	Group IV Severe	
Obese	With	0	0	1	2	3
	Without	1	7	5	8	21
Average	With	4	1	2	3	10
	Without	7	5	9	10	31
Poor	With	8	7	8	3	26
	Without	6	6	1	0	13
Totals	With	12	8	11	8	39
	Without	14	18	15	18	65

weight loss, had moderate or severe formation of hyaline and calcific plaques.

The finding that both lipid deposits and hyalinized and calcified plaques bear the same relationship to the general state of nutrition is further evidence that these lesions, as commonly believed, are related to one another, and that hyalinization and calcification occur in areas that were originally the site of simple lipid deposits.

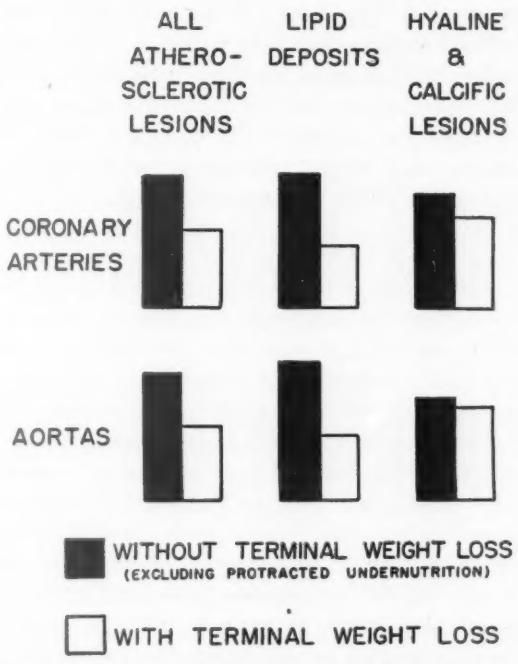
In Text-Figure I the incidence of moderate and severe atheromatous lesions of various types in those who had lost weight terminally and in those who had not is contrasted graphically.

The Relation of Terminal Weight Loss to the Histologic Appearance of Atheromatous Lesions

Histologic sections of Zenker's-fixed, paraffin-embedded tissues stained with hematoxylin and eosin were studied from most of the aortas and many of the coronary arteries used in this analysis. Since in any one artery the lesions are likely to vary considerably in character and distribution, random sections provide an inadequate sample of

the atherosclerotic process as a whole. It is, therefore, not possible to present quantitatively the histologic differences observed. In general, however, the gross observations were confirmed, namely, that the atheromatous lesions were less pronounced in those who had lost weight terminally than in those who had not. This was true of almost all features of the process but more particularly in respect to lipid deposition.

Lipids can be identified in atheromatous lesions chiefly in three



Text-Figure 1. The incidence, in percentage, of moderate and severe atheromatous lesions of various types in the aortas and coronary arteries of persons who had not lost weight prior to death as compared with those who had lost weight terminally.

forms: contained within phagocytes, as cholesterol crystals, and impregnating diffusely the amorphous material in the depths of large intimal plaques. As a rule, less lipid in each of these three forms was noted in the arteries of those who had lost weight terminally than in the arteries of those who had not. The most consistent difference, however, was in the number, size, and distribution of lipid-containing phagocytes. In the group with wasting disease these were generally few in number, relatively small, and often scattered as single cells in crev-

ices of dense or hyalinized connective tissue. In the group without terminal weight loss, these cells were often numerous, grouped in clusters, swollen with numerous fine droplets of fat, and superficially located just beneath the endothelial surface or at the margins of large intimal plaques. These differences were by no means constant or uniform. Cholesterol crystal spaces were found less often in the amorphous material contained at the base of large intimal plaques in those with terminal weight loss than in those without terminal weight loss. The amount of amorphous material deposited in the depths of intimal plaques was also generally less. On the other hand, diffuse intimal thickening without discrete plaque formation was often more conspicuous in those who had lost weight terminally. No conspicuous difference in the amount of hyalinized intimal connective tissue was noted, although other regressive changes such as calcification, "ulceration," chronic inflammation, increased vascularity, and hemorrhage were less often observed in the group with terminal weight loss.

Discussion

There is a widely held belief that dietary factors are concerned in the development of human atherosclerosis, but this belief is not based on very tangible evidence. Rosenthal,[11] however, has compiled considerable data which indicate that a high rate of atherosclerosis is found among consumers of high-fat diets. An analogy between atheromatosis in cholesterol-fed rabbits and in man is hardly admissible since cholesterol feeding does not lead to marked, persistent hypercholesterolemia in man according to Hueper,[10] who has summarized the abundant, somewhat contradictory literature on this subject. While in a few instances persistent hyperlipemia or hypercholesterolemia is apparently associated with atherosclerosis in man, as in diabetes mellitus, multiple xanthomatosis, myxedema, and lipid nephrosis, in the large majority of cases such an association has not been demonstrated. In any event, dietary factors probably are not primarily concerned in any of these hyperlipemic states.

The state of nutrition in health and to a large extent in wasting disease is dependent on food consumption. The results of the present study indicate that the general state of nutrition is a factor not only in the development but also in the resolution of human atherosclerotic lesions. It may be inferred, therefore, that a dietary influence not necessarily associated with persistent hyperlipemia is involved in the pathogenesis of human atherosclerosis.

Since obesity is not invariably associated with severe atherosclerosis, and terminal weight loss is not always followed by appreciable resorp-

tion of lipid deposits, the state of nutrition may be only a secondary and subsidiary factor that becomes effective only if more fundamental, predisposing conditions are fulfilled; or only specific constituents in the diet may be concerned. For example, overnutrition due to high-fat or high-cholesterol diets may lead to atherosclerosis more readily than overnutrition due to high carbohydrate intake. The infrequent association of obesity and atherosclerosis in young persons and, in older age groups, the less constant association of the two in women than in men [1] argue against the explanation that specific items of food consumption are primarily involved. It is conceivable that both nondietary, predisposing factors and the consumption of specific food materials in abundance are necessary to promote the development of atherosclerotic lesions.

It is frequently assumed that atherosclerosis is a slowly evolving, continuously progressive process. The implication of the findings reported here is that lipid deposits may be withdrawn from arterial deposits in relatively short periods of time. By inference, it is equally logical to assume that during periods of rapid gain in weight new deposits of lipid may form with equal rapidity. The atherosclerotic process may well progress and recede rapidly and intermittently, at least during the early stages of its development. The amount of lipid found in the intima at the end-stage may represent only a minute fraction of the total amount of lipid that has penetrated the vessel wall. The not uncommon discrepancies in evidence of generalized atherosclerosis as observed clinically and the degree found at necropsy are partly explainable on the basis of probable fluctuations in the anatomical manifestations of the disease.

Summary and Conclusions

A high incidence of severe atherosclerosis is found in obese persons at necropsy. Severe or even moderate atherosclerotic change is seldom observed in those with protracted undernutrition. When the group with terminal weight loss is not omitted from the analysis, a correlation between the state of nutrition and degree of atherosclerosis, although much less pronounced, is still demonstrable. When the analysis is limited to the degree of hyalinization and calcification of intimal plaques, inclusion of the group with terminal wasting disease almost totally obscures the relationship between nutrition and these features of the atherosclerotic process.

Less severe degrees of atherosclerotic change are usually observed in the group with wasting disease than in those without terminal weight loss. It is inferred, therefore, that significant resorption of previously

formed atheromatous lesions may occur during periods of marked weight loss. The most conspicuous and consistent difference in the two groups is observed in the amount of lipid contained in the intimal lesions. In general, less lipid, and in particular, fewer lipid-containing phagocytes are demonstrable in the intimal lesions of those with terminal weight loss. Less constant and significant differences are observed in other features of the atherosclerotic process in the two groups. It is suggested, therefore, that the early lesions are the ones that are most susceptible to regression during periods of weight loss.

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TWO SIMULTANEOUS CASES OF LEPROSY DEVELOPING IN TATTOOS *

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Though leprosy is one of the oldest known diseases, there is still uncertainty and confusion concerning its transmission and the incubation period. Close association with lepers over a period of years has always been a recognized source of danger, and yet the few who developed leprosy, such as Father Damien, are notable exceptions. Minimal precautions seem adequate to prevent the spread of leprosy. Various theories as to transmission have enjoyed temporary popularity. Among the more intelligible of these were the concept of fish as intermediate hosts, the idea that wearing clothing discarded by lepers produced the disease, that person to person vaccination spread leprosy, that various insects acted as vectors, that sexual intercourse was responsible, and that leprosy was acquired through the nasal mucous membrane.

During the past 100 years, one of the most debated questions has been whether or not leprosy can be spread by the inoculation of tissue or other contaminated material from a leper into the skin of an uninfected person.

Jeanselme,[1] in 1934, concluded that there was no adequate proof of the transmission of leprosy by inoculation. He cited the experiments of Danielssen and Boeck,[2] Profeta,[3] and Mouritz [4] during the 19th century. Danielssen repeatedly inoculated himself but never showed evidence of leprosy. Profeta inoculated 10 persons experimentally without reproducing the disease. Mouritz likewise got negative results.

Klingmüller,[5] in 1930, gave a good review of the evidence for and against the experimental inoculation of leprosy.

Rogers and Muir,[6] in 1940, specifically accepted the transmission of leprosy by inoculation and considered it an important factor. They described, but questioned, the case of Keanu who was inoculated by Arning,[7] in 1886, in the Hawaiian Islands. He subsequently developed leprosy and died. Unfortunately, Keanu came from a leprous family and also lived in close contact with lepers. Cases accepted as valid evidence of leprosy developing after inoculation include that of Marchoux [8] who, in 1922, while operating on a leper, pricked the finger of his assistant who developed leprosy after several years. De Langen,[9] in 1931, reported the accidental inoculation of a patient by a physician who confused his syringes after giving a hypodermic to a leper. A leprous nodule developed at the site of the injection in 6 months. La-

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goudaky,[10] in 1936, was repeatedly injected with blood from a leper. In less than 2 months after his first inoculation he developed cutaneous lesions of leprosy.

Negative results from experimental inoculations are difficult to evaluate because of the long incubation period and the possibility of subclinical or dormant infections as in tuberculosis. There is a wide variation of susceptibility to the disease among persons, between the sexes, in different age groups, and among races. Since leprosy tends to localize in scars and tattoos, and to be activated by trauma, it is difficult to prove that an infection following inoculation actually resulted from it.

The remarkable coincidences in the 2 cases to be presented offer very strong evidence favoring the transmission of leprosy by inoculation. Both men were members of the same unit in the United States Marine Corps. They were tattooed successively by the same man in Melbourne, Australia, on the same day in June, 1943. Both developed maculo-anesthetic leprosy in the tattoos about 2½ years later. Both the Marines and the tattooer were inebriated and a number of needles were broken during the process. One man had multiple tattoos on his left arm but developed leprosy only in the one made in Melbourne. The two men in civil life are friends and residents of the same town, and one was instrumental in bringing the other to the doctor for diagnosis. A third man tattooed at the same place, but at a different time, as yet shows no evidence of leprosy.

Case 1

The patient, W. J. H., was a white male, 24 years of age, whose chief complaint was that of increased pigmentation and numbness of a tattoo on the back of the left forearm.

Present Illness. In June, 1943, the patient, while in company with L. G. (case 2), was tattooed on the extensor surface of the lower left forearm in Melbourne, Australia. Nothing took place to suggest any untoward development in the tattoo until March or April of 1946 when the patient noticed that the area of the tattoo and a zone about 1.5 cm. in width about it had become pale red (Fig. 1), and was insensitive to light touch and pain. During June, 1946, while at a party, he deliberately burned himself in this lesion with a lighted cigarette to prove the loss of sensation.

Past Illness. The patient had had the usual childhood diseases. He had developed malaria in December, 1942. The left scapula had been chipped in an accident during September, 1945, and he has noted some weakness in his left arm since that time.

Family History. The family history was not significant.

Physical Examination. The patient's temperature was 98.6°F.; pulse, 76 per minute; blood pressure, 100/60 mm. Hg. On routine physical examination a slight congestion of the nasal mucous membrane was noted and the left upper arm was found to be 8 mm. less in circumference than the right arm. The axillary and inguinal lymph nodes were small. There was a nontender swelling on the lateral surface of the juncture of the middle and lower thirds of the left upper arm. This

measured about 0.5 by 4 cm. There was no palpable evidence of lesions of the nerve trunks. The reflexes were normal. There were two tattoos on the extensor surface of the left forearm. About the distal one (made in Melbourne, as previously noted) there was a uniform, pale fawn-colored area involving the entire tattoo and a narrow zone about it. The total diameter was 9 cm. There was a loss of sensation to pain and light touch in the discolored area. There were three small depressed scars in the center, each about 0.5 cm. in diameter. The other tattoo showed no evidence of disease.

Laboratory Examination. Laboratory findings were as follows: The Kahn reaction of the blood was negative. Hemoglobin was 16 gm.; red blood cell count, 4,630,000; white blood cell count, 6,300; differential count, 46 per cent polynuclear neutrophils with 37 per cent segmented cells, 7 per cent stab cells, and 2 per cent juveniles, 7 per cent large and 45 per cent small lymphocytes, 8 per cent eosinophils, and 1 per cent monocytes. Routine chemical and microscopic urinalyses were normal.

On November 2, 1946, tissue was taken for biopsy from the pigmented area outside the tattoo. The excision was made without benefit of anesthetic and caused the patient no discomfort. The microscopic sections showed a tuberculoid reaction, and occasional acid-fast bacilli, averaging about four per section, were found by the Ziehl-Neelsen method. Smears from the nasal mucous membrane were negative for acid-fast bacilli. Intradermal inoculation with 0.1 cc. of O.T. (1: 10,000) was positive. In view of the history and microscopic findings, a provisional diagnosis of cutaneous tuberculoid leprosy was made.

Case 2

L. G. was a white male, 25 years old, whose chief complaint was numbness and pigmentation of a tattoo on the flexor surface of the left forearm.

Present Illness. In June, 1943, the patient, in company with W. J. H. (case 1), was tattooed on the flexor surface of the left forearm in Melbourne, Australia. About January, 1946, he noticed that the area of the tattoo and a zone about 1.5 cm. in width about it was becoming dusky red (Fig. 2) and numb. Since then the color had gradually darkened. Two new areas (Fig. 3) had appeared over the triceps of the upper left arm 7.5 cm. above the elbow. These became confluent. They were a dark violaceous color and were numb. There was no elevation of the skin surface. The patient's general health remained good.

Past Illness. The patient had had the usual childhood diseases and had developed malaria in December, 1942. He had had occasional malarial chills since.

Family History. The family history was not pertinent.

Physical Examination. The patient's temperature was 99°F.; the pulse rate, 72 per minute; blood pressure, 120/70 mm. Hg. Routine examination of the head, neck, chest, abdomen, and genitalia showed no abnormality. No surgical scars were present. In the left lumbar region at the waistline there was a violaceous, flat lesion 1 cm. in diameter. This had normal sensation and was not definitely related to the present illness. The axillary and inguinal lymph nodes were normal in size. On the flexor surface of the left forearm there was a tattoo. The skin of the entire tattoo and a zone 1.5 cm. in width about it showed a violaceous discoloration. There was a loss of sensation of pain and to light touch throughout the entire pigmented area. There was no ulceration or elevation present. On the extensor surface of the left upper arm 7.5 cm. above the elbow there were two coalescent lesions making an hour-glass-shaped area about 2.5 by 4 cm. This had the same

violaceous color as the tattooed area and there was the same loss of sensation. There was no palpable abnormality of the nerve trunks.

Laboratory Examination. Laboratory findings were as follows: The Kahn reaction of the blood was negative; hemoglobin was 18.6 gm.; red blood cell count, 5,110,000; white blood cell count 6,500; differential count, 45 per cent polynuclear neutrophils with 41 per cent segmented cells, 3 per cent stab cells, and 1 per cent juveniles, 7 per cent large and 32 per cent small lymphocytes, 14 per cent eosinophils, and 2 per cent monocytes. The routine chemical and microscopic urinalyses were normal.

On November 11, 1946, a specimen was taken for biopsy from the pigmented area outside the tattoo. No anesthetic was necessary due to the lack of sensation of pain. The tissue was divided into two parts. One was sent in saline solution to the Michigan Department of Health. The report received stated that no acid-fast bacilli were found in direct smears or in culture. Two guinea-pigs inoculated with the tissue taken for biopsy showed no evidence of tuberculosis after 7 weeks.

The other part of the tissue was fixed in formalin and embedded in paraffin. The microscopic appearance was the same as in case 1. Acid-fast bacilli were demonstrated by the Ziehl-Neelsen method, but they were less common than in case 1 and averaged only about one per section. Smears from the nasal mucous membrane were negative for acid-fast bacilli. Intradermal inoculation with 0.1 cc. of O.T. (1: 10,000) was negative. The tentative diagnosis was cutaneous tuberculoid leprosy.

The first case was informally described to Dr. Claude Behn of Detroit, who, without seeing the patient, made the original suggestion of leprosy as a probable diagnosis.

Unstained sections from both cases were submitted to the United States Public Health Service. From the U.S. Marine Hospital (Leprosarium) at Carville, Louisiana, came an unequivocal diagnosis of "typical tuberculoid leprosy," but acid-fast bacilli were not demonstrated.

These lesions could well be tuberculous as far as the gross appearance is concerned. Microscopically, the presence of epithelioid tubercles with Langhans' giant cells, lymphocytic and plasma cell infiltration, and occasional acid-fast bacilli are as characteristic of tuberculosis as of leprosy. The history is more suggestive of leprosy than tuberculosis. The loss of sensation to pain and light touch in the pigmented lesions, the presence of a positive tuberculin skin test in case 1 and a negative test in case 2, the failure of guinea-pigs to develop tuberculosis after inoculation with tissue containing the acid-fast bacilli, the failure to culture acid-fast bacilli, the presence of vacuolated cells, and the positive diagnosis received from the U.S. Marine Hospital at

Carville, Louisiana, establish these as cases of maculo-anesthetic or tuberculoid leprosy.

The long incubation period suggests resistance to the disease on the part of the patients. The extensive traumatization of the skin incident to tattooing might favor the development of the disease. It is possible that the multiple skin punctures led to massive inoculation, but that is purely speculative.

It is of interest that, as has been noted by other observers, cinnabar (red mercuric sulphide) in the tattoos, which discourages spirochetal activity in syphilis of the skin, does not have any apparent effect on the bacillus of leprosy.

The tissues taken for biopsy from the two patients were so similar that a single description will suffice. In each case the specimen was taken from the pigmented lesion, near its edge, outside the tattoo. Gross examination of the tissue showed a smooth skin surface with moderate pigmentation and no ulceration. The microscopic appearance (Figs. 4 to 8) of the tissue so closely resembled tuberculosis of the skin as to be almost, if not quite, indistinguishable. Many of the tubercles suggested Boeck's sarcoid but occasional ones showed an appreciable degree of caseous necrosis. The Langhans' giant cells were of all sizes and their appearance and distribution were no different from those of tuberculosis. They occurred both in the epithelioid foci and scattered through the areas of lymphocytic infiltration. The characteristic lesion was tuberculoid in type. It consisted of a center of epithelioid cells with a rim of lymphocytes, a few plasma cells, and even fewer polymorphonuclear cells. Occasional eosinophilic leukocytes were present, at times infiltrating between the epithelioid cells. The nodule was largely avascular. The largest showed some central caseous necrosis but this was not common. The tuberculoid foci were present throughout the tissue specimen, both in the corium and subcutaneous fat. The process apparently extended beyond the depth of the excised tissue.

The epidermis was irregularly atrophic and there was flattening and partial loss of the dermal papillae. In some areas there was lymphocytic invasion of the basal and prickle cell layers. There was no tendency to epithelial overgrowth, such as occurs in blastomycosis.

The hair follicles had lymphocytic infiltration about and in them and the picture was entirely compatible with the loss of hair characteristic of lepromas. There was a granulomatous involvement of the sweat glands, some of which had almost completely disappeared, being replaced by epithelioid nodules with lymphocytes and plasma cells.

The largest tuberculoid foci were present in the deep layer of the

corium, with smaller nodules and extensive lymphocytic infiltration in the superficial layer. There were no characteristic leprous foam cells, which usually contain large numbers of acid-fast bacilli, but there were occasional vacuolated cells which were suggestive.

The tuberculoid foci in the subcutaneous fat were more discrete than those nearer the epithelial surface and there was no generalized involvement of the adipose tissue. Here the tubercles were smaller than those in the deep layer of the corium.

The cutaneous nerves were involved but not more so than other structures. There was no particular evidence in these tissues that the process was extending by way of the nerves. There was quite extensive involvement of the small vessels but they did not show the swelling and proliferation of the endothelium which is found in syphilis.

Ziehl-Neelsen staining of the sections showed occasional acid-fast bacilli in the first case and rare ones in the second. The bacilli were found most often in or about the largest tuberculoid lesions in the deep layer of the corium. Usually they occurred in pairs or with two single organisms in one oil-immersion field. The acid-fast bacilli showed no significant variation from tubercle bacilli either in morphologic characteristics or staining qualities.

Summary

Two men from the same community, while serving in the United States Marine Corps, were tattooed by the same man on the same day in June, 1943, at Melbourne, Australia. They both developed maculoanesthetic or tuberculoid leprosy in their tattoos during the first half of 1946. One man had multiple tattoos but developed leprosy only in the tattoo made in Melbourne the day when his friend was tattooed. A third Marine, tattooed at the same place but not on the same day, has shown no evidence of leprosy. These two cases provide strong evidence for the spread of leprosy by inoculation.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 127

FIG. 1. Case 1. The smaller distal tattoo made in Melbourne is the only one with pigmentation and anesthesia. The pigmentation is so light that it does not appear in the photograph. The dark spot by the star indicates the site where tissue was excised for biopsy.

FIG. 2. Case 2. Tattoo on the left forearm showing the extent of the pigmentation. The skin suture is still present where tissue was taken for biopsy.

FIG. 3. Case 2. Secondary lesions on the extensor surface of the left upper arm.

FIG. 4. Case 2. Large Langhans' giant cell and a small epithelioid tubercle with lymphocytic infiltration about them. Hematoxylin and eosin stain. × 500.

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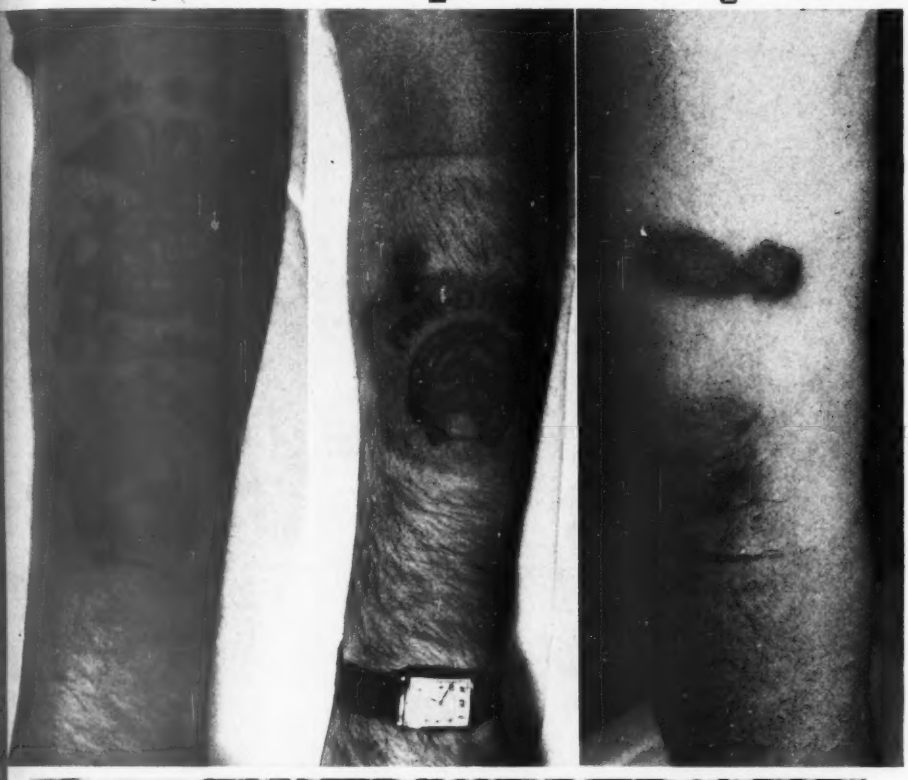

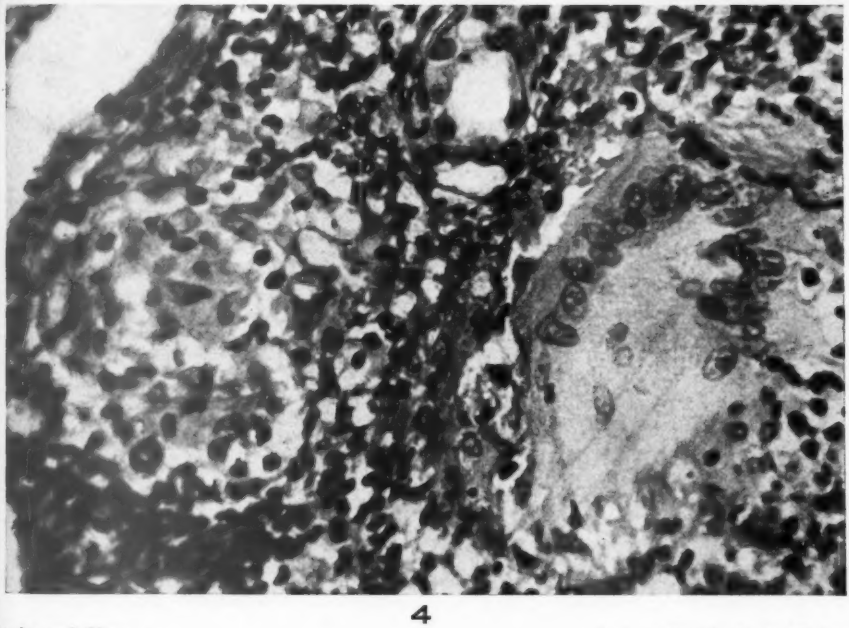

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Porritt and Olsen Leprosy Developing in Tattoos

PLATE 128

FIG. 5. Case 1. Lepromatous reaction about a sweat gland. Hematoxylin and eosin stain. × 500.

FIG. 6. Case 1. Lymphocytic infiltration of the epithelium and corium with a well formed Langhans' giant cell. Hematoxylin and eosin stain. × 500.

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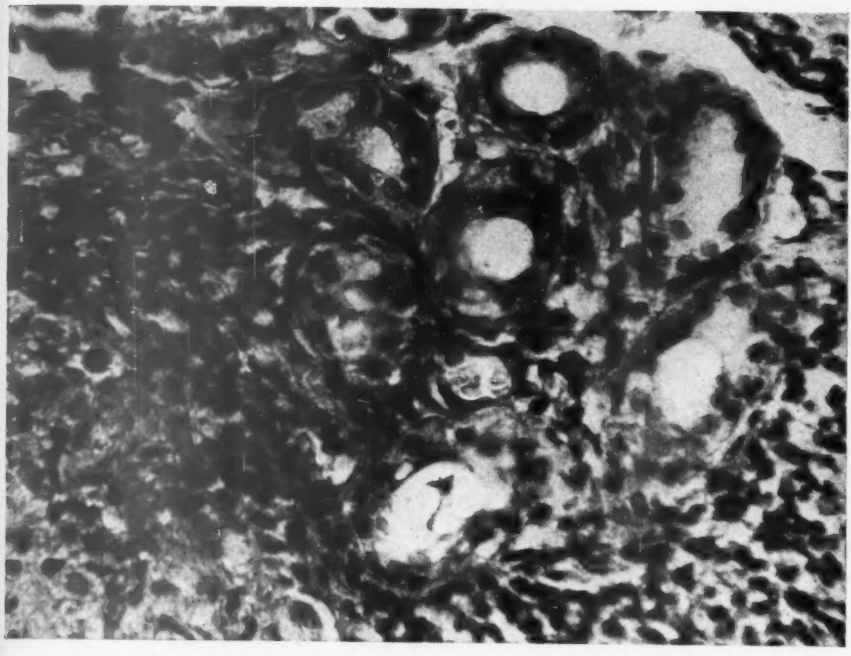
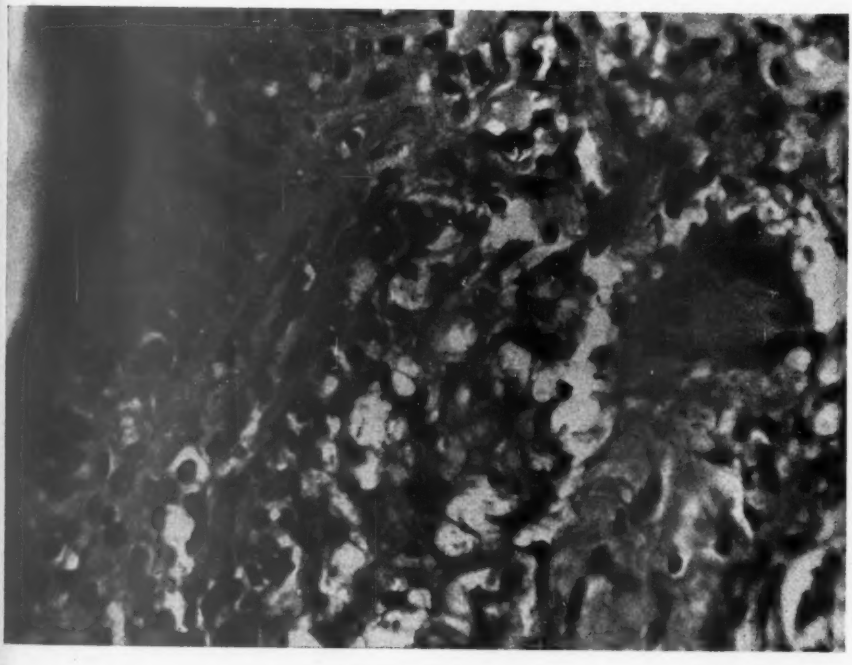


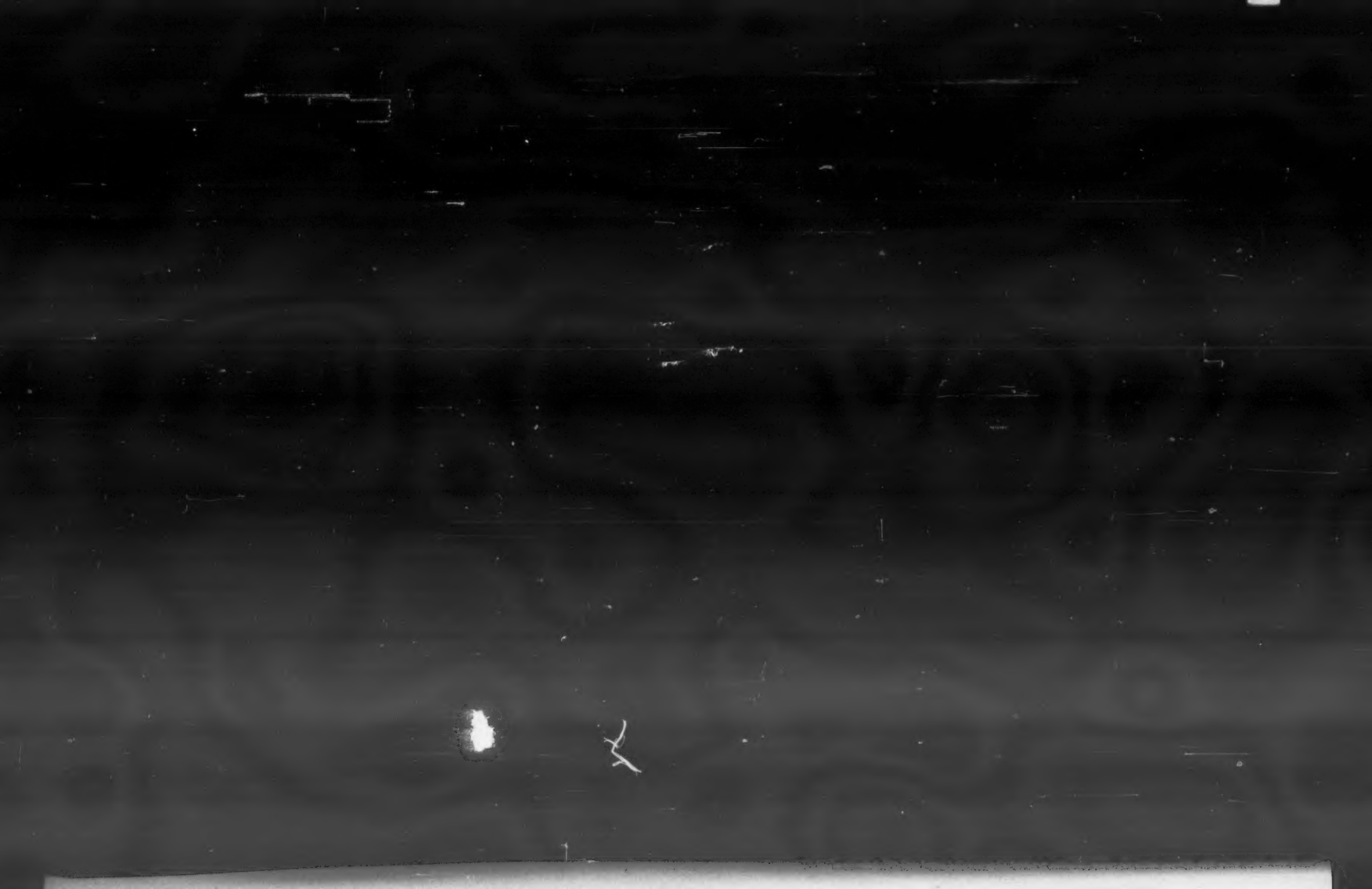


Porritt and Olsen Leprosy Developing in Tattoos

PLATE 129

FIG. 7. Case 1. Lymphocytic infiltration in and about a hair follicle. Hematoxylin and eosin stain. × 500.

FIG. 8. Case 2. Large epithelioid tubercle with caseous necrosis in the center. This lesion is microscopically indistinguishable from tuberculosis. Hematoxylin and eosin stain. × 180.



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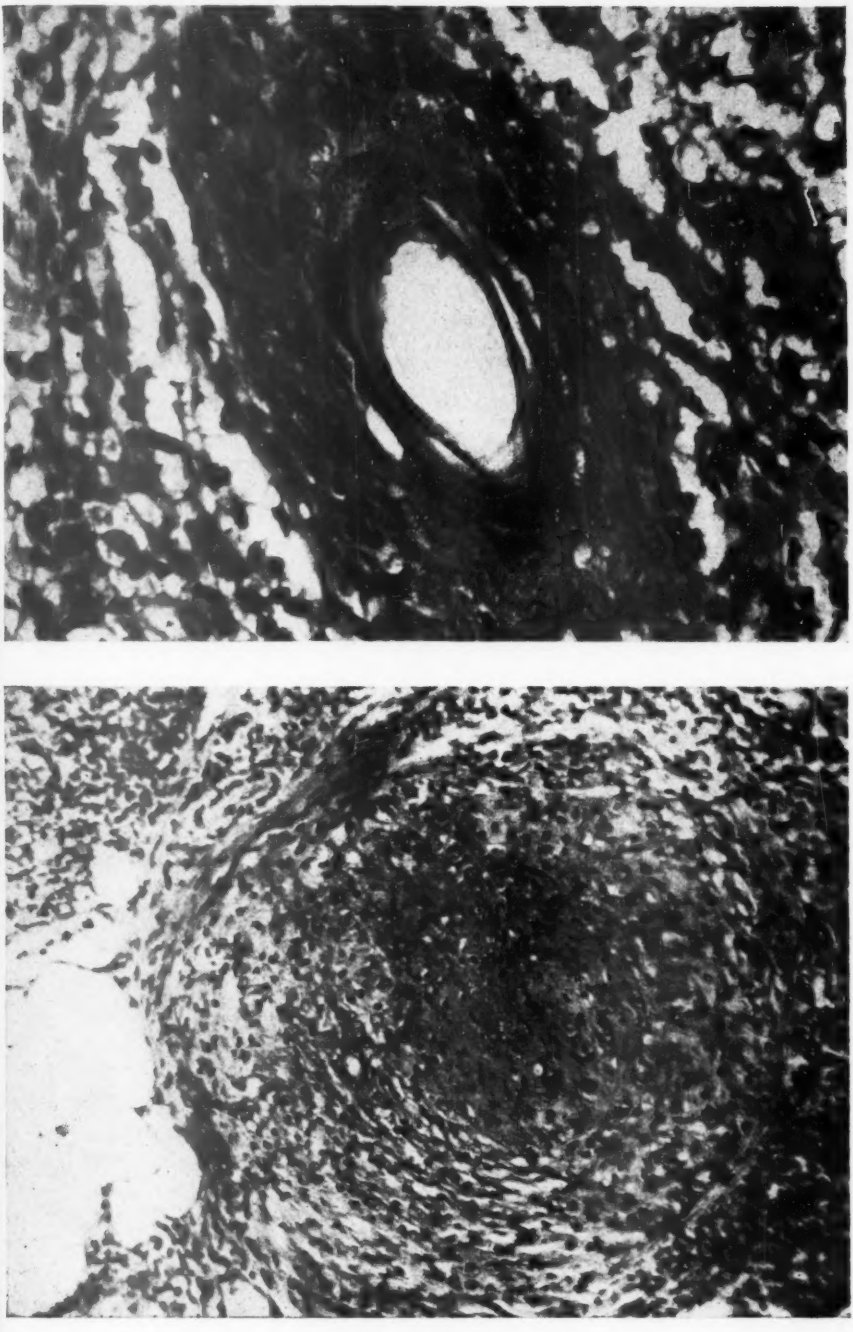

Porritt and Olsen Leprosy Developing in Tattoos

THE INTESTINAL PHASE OF HUMAN TRICHINOSIS *

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Study of the tissues of fatal cases of trichinosis in man has been concerned largely with the lesions in muscle, heart, and brain produced by the larval form of the parasite. Discovery of adult worms in the intestine has been reported in only a few instances,[1-7] and in some of these reports this observation has not been confirmed by microscopic examination. The presence of gravid females is of major clinical importance since from them come the larvae which cause the muscular, cardiac, and cerebral lesions. Determination of the maximal duration of the intestinal phase in the human host can be made only by observations upon human material, since in each species the factors influencing the duration are different. Even within a species the duration of the intestinal phase will be influenced by such factors as the extent (heaviness) of the infestation and the prior contact of the host with the parasite.

The duration of productivity of the adult female trichina is generally stated to be about 6 weeks. This figure is based chiefly on experimental work with animals, especially that of Roth [8] with guinea-pigs. In these few reported instances in which the presence of adult worms in the human intestine has been confirmed by microscopic examination, the longest duration of the infection was 30 days [6]; in one report [9] of a patient dying on the 56th day of illness it is stated that no parasites could be found in the intestine.

REPORT OF CASE

In the necropsy service of the Department of Pathology of the University of Michigan a case of trichinosis has been observed in which many living adult worms were demonstrable in the intestine. The patient was a tavern keeper, 35 years old, who had eaten raw pork sausage prepared from a hog he had raised and butchered. Other members of his family and neighbors who had eaten the raw meat likewise developed trichinosis, and the disease proved fatal also to an 8-year-old daughter. It was stated positively by both the patient's wife and the family physician that the raw sausage had been consumed on only one occasion. The father and the daughter ate large amounts of the meat. The clinical course of the father was characterized by diarrhea, marked muscular pain, fever, and terminal respiratory distress. There were

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physical signs and electrocardiographic findings indicating myocardial damage and neurologic abnormalities suggesting cerebral damage. The patient was hospitalized for the last 4 weeks of life. Death occurred on the 54th day after the eating of the raw pork and sausage.

The gross examination of the intestines at the time of autopsy revealed only scattered petechial hemorrhages in the mucosa. No parasites were seen. A portion of the bowel was fixed unopened so as to preserve the contents.

On microscopic examination, adult trichinae were found in many sections of both the large and small intestine; they were most numerous in the jejunum. Both male and female worms were present. In the latter, larval forms could usually be recognized. Adult worms were more numerous in the sections made from unopened intestine, where the contents of the lumen were undisturbed. In single sections the trichinae could be found lying free in the lumen of the intestine (Fig. 1) or embedded in the mucosa (Fig. 2). When traced by serial sections a worm was frequently found to have one end or its mid-portion embedded in the mucosa, while the remainder extended into the lumen. In some areas the mucosa completely enclosed a portion of the adult worm. Most frequently it was the uterine area that was embedded in, or in contact with, the mucosa. Occasionally a larval form could be found lying in the mucosa adjacent to an adult. There was no significant cellular reaction to the parasites within the mucosa. Lymphocytes, plasma cells, and eosinophils were found near worms; but such cells were present equally in the mucosa distant from the embedded parasites and in control sections from other cases in which no parasites were found. There were no areas of granulomatous inflammation and no foci of necrosis or hemorrhage. The mucosal glands and connective tissue near a worm were compressed by it; these elements were directly in contact with the cuticle with no surrounding area of lysis. One adult trichina was found to have penetrated the mucosa to the level of the muscularis mucosae. No adult worms or larvae were found external to the muscularis mucosae.

Sections of skeletal muscle from many areas were examined and in all was an unusually heavy parasitization by larval trichinae (Fig. 3). Even in the fibers of the cremaster muscle along the spermatic cord many larvae were found. Most of the larvae were coiled and formation of the wall of the cyst had begun. A few larvae were still straight. Single fibers contained as many as three larvae at the level of a single section. Many muscle fibers showed hyaline degeneration, and there was a heavy cellular infiltration between the muscle fibers; the infiltration was composed of mononuclear cells of macrophagic type, lympho-

cytes, plasma cells, and many eosinophils. In a few foci the exudate was purulent. Quantitative examination of the diaphragm for content of trichinae was performed by Dr. S. E. Gould.* Each gram of diaphragmatic muscle from near its tendinous attachment, examined by the digestion method, revealed an average of 2677 larvae. This is one of the heaviest infestations that has been reported. Examination of the heart and the brain revealed typical trichinous myocarditis [10] and encephalitis.[5, 11] Larvae were demonstrable in each of these organs (Fig. 4).

Discussion

The length of time living adult trichinae remain in the intestinal canal is related to three factors: the species of animal parasitized, the state of immunity present in the host, and the heaviness of the infestation. These three factors are closely correlated, and in each case all are effective.

Trichinella spiralis is able to parasitize a wide range of hosts, chiefly mammalian. There is a strong correlation between the natural resistance of the host to the lethal effects of generalized trichinosis (natural immunity) and the length of time the worms remain in the intestine. This is exhibited by a reciprocal relationship. The more tolerant or resistant the host to the effects of larval trichinae, the shorter the time that living adults can remain in the intestine. The dog has been found to be relatively resistant to infection; live adult worms have been seen in the canine intestine for only 10 days after feeding.[8] In the gopher, relatively susceptible to the parasite, intestinal trichinae have been found as long as 13 weeks after infection.[12]

The intestinal phase of the life cycle of *T. spiralis* has also been demonstrated to be related to acquired immunity. Immunity against re-infection by the parasite was shown by Ducas [13] to be localized in the intestines, and this conclusion has been confirmed in numerous subsequent investigations.[14–21] The mechanism of acquired immunity depends apparently on retardation of the development of ingested larvae to adult worms, plus a deleterious effect upon any adult worms present.[14, 18, 22] In immune rats there has been observed a rapid loss of larvae from the intestines 8 to 18 hours after feeding.[23]

A relation between the number of infecting larvae and the time during which adults could be found in the intestine of rats was shown by McCoy.[24] In light infections the adults could be recovered for only 14 to 16 days. With heavier infections, adults could be found for 4 to 5 weeks. With massive infection, death resulted in 2 weeks from the effects of the intestinal phase. In the guinea-pig, Roth[8] was not able

* Wayne County General Hospital, Eloise, Michigan.

to demonstrate a correlation between infecting dose and duration of the intestinal phase.

The results of animal experimentation lead to conclusions which are applicable to observations of human trichinosis. Man is relatively susceptible to trichinosis, and thus the intestinal phase may be prolonged. In the first contact of the host with the parasite acquired immunity does not develop, and this also tends to cause a prolonged intestinal phase. In a second infection in man, the adults should have a shorter life. The number of larvae ingested is relatively small in most persons. The "dilution" of pork from an infected pig by combining it with meat from noninfected animals increases the number of persons infected but decreases the extent of the infection in each individual. However, when an infected animal is the sole source of meat, the infection may be heavy and a long intestinal phase may result.

In the case here reported, the infection was a first infection (all muscular lesions were recent) and the quantity of larvae ingested was great. Thus the long intestinal phase in this patient is in accord with the conclusions obtained from animal experimentation.

The significance of a prolonged intestinal phase in trichinosis is obvious. Persistence of systemic myositis and increased severity and duration of the characteristic myocarditis and encephalitis result. Death during the acute phase of trichinosis is usually due to the lesions of the heart and central nervous system. Pulmonary embolism and infarction frequently seen in fatal trichinosis may also be related to the number of larvae, in that immobilization by the pain of muscular movements favors hemostasis with resulting thrombosis and embolism. The heart and the brain are affected only while the larvae are in a migratory state, since no encystment occurs in these organs. With disappearance of the larvae the inflammation subsides. These facts were clearly illustrated in the case here presented. Well formed larvae were demonstrable in both the heart and the brain, and there was granulomatous inflammation of these organs. The clinical course included symptoms indicating both myocarditis and encephalitis. The discovery of adult worms in the intestine and the presence of larvae within adult females showed that release of larvae was still occurring at the time of the patient's death, 54 days after eating the infected meat.

These observations emphasize the importance of therapeutic efforts directed against the adult trichinae in the intestine, either by the use of purgatives or of immune or convalescent serum. There is evidence that many of the adults can be removed by purgation.[25, 26] Immune or convalescent serum is believed to act upon the ingested larvae which

are maturing in the intestines and thus decrease the number of effective adults.[22] There is no evidence that anti-helminthic drugs are of value.[27] Purgation, with careful attention to fluid balance and the general state of the patient, at present offers the most hope for reducing the number of gravid female worms.

Summary

Living adult trichinae, including gravid females, were demonstrated in the intestine of a fatal case of human trichinosis 54 days after ingestion of infected pork. This is the longest period of persistence of adult trichinae in the human intestine thus far reported, with microscopic demonstration of the adult parasites *in situ*. The possibility of continued release of larvae over a period of even greater length must be taken into account in the therapeutic management of trichinosis.

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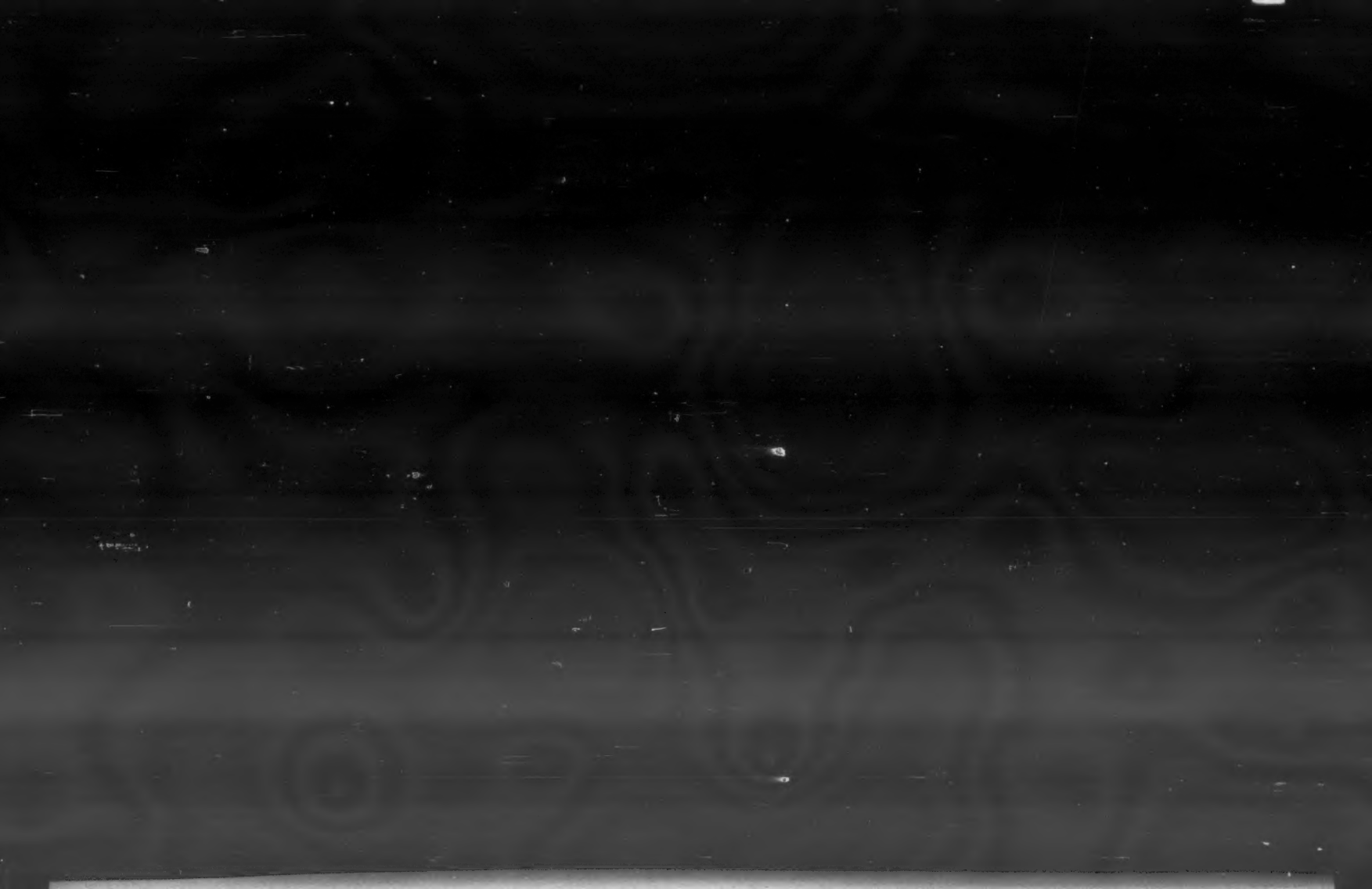
DESCRIPTION OF PLATES

PLATE 130

FIG. 1. Adult female *Trichinella spiralis* in small intestine. Larvae can be seen within the uterus. Hematoxylin and eosin stain. × 200.

FIG. 2. Cross section of an adult female *Trichinella spiralis* in the mucosa of the small intestine. Numerous larvae are present within the uterus. Hematoxylin and eosin stain. × 600.



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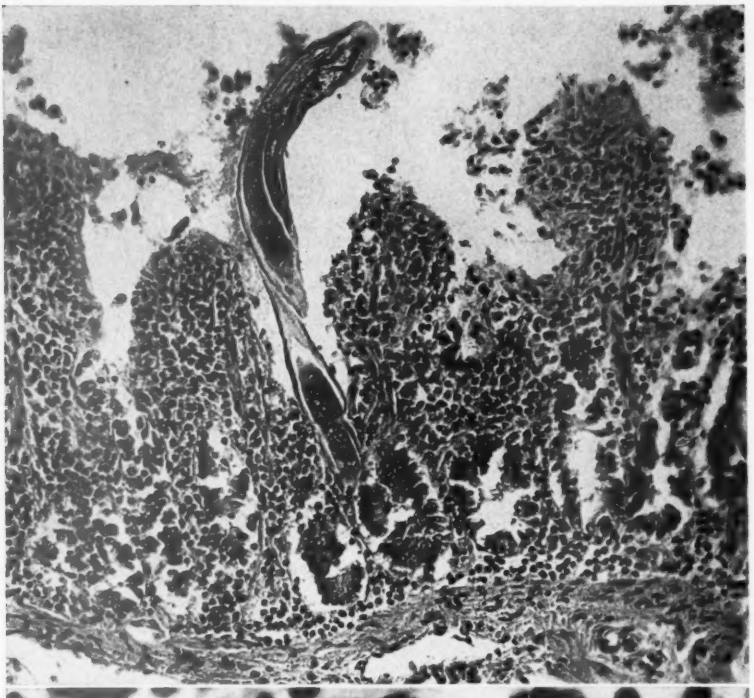

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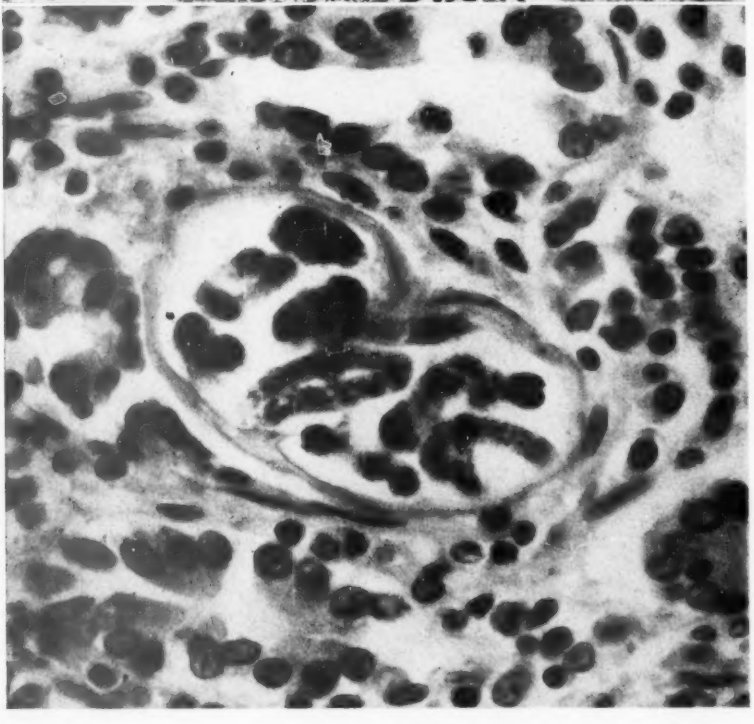

Stryker Intestinal Phase of Human Trichinosis

PLATE 131

FIG. 3. Encysted *Trichinella spiralis* in the diaphragm. Hematoxylin and eosin stain. × 150.

FIG. 4. Larval *Trichinella spiralis* in brain. The larva in upper center of field lies in a small granuloma. Hematoxylin and eosin stain. × 800.

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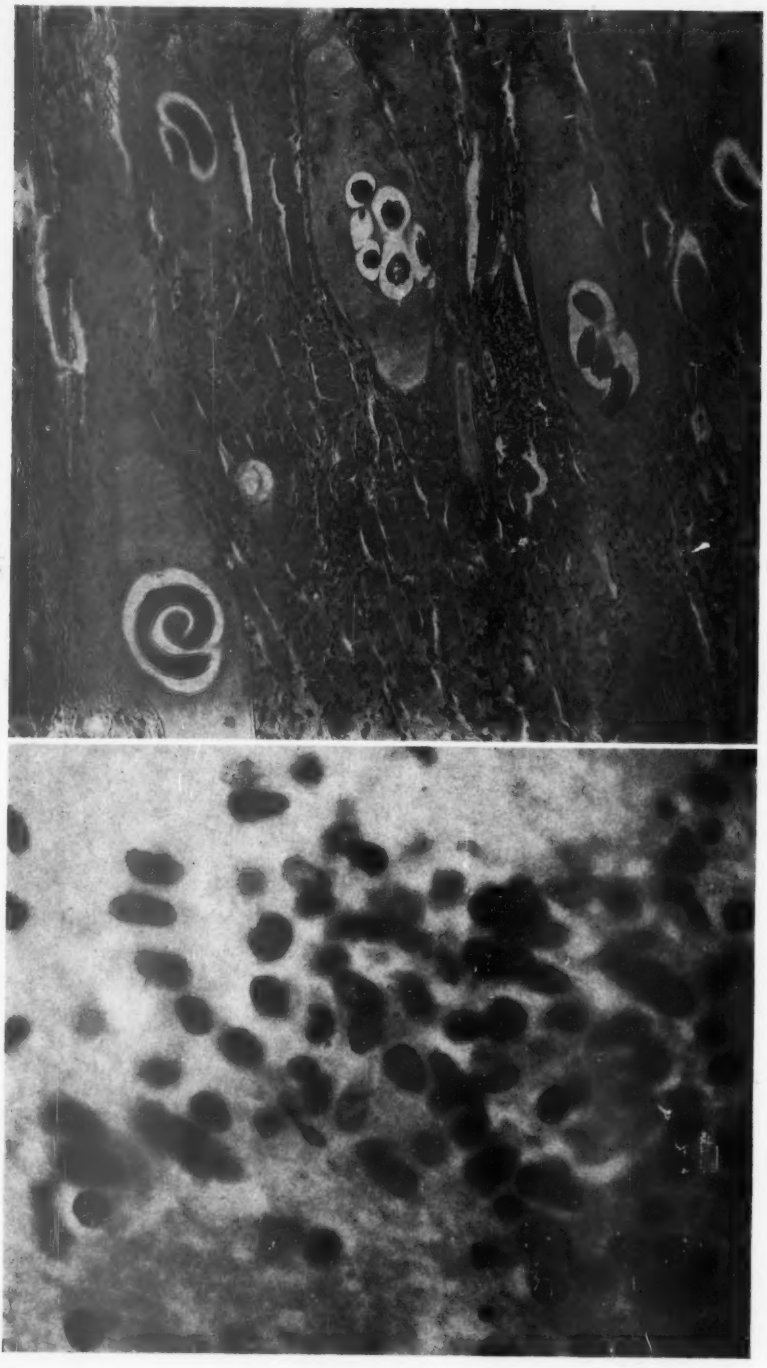

Stryker **Intestinal Phase of Human Trichinosis**

STUDIES ON CAPILLARY PERMEABILITY AS AFFECTED BY ANOXEMIA *

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The endothelial lining of blood vessels and lymphatics, especially that of capillaries, can be considered as a membrane which acts in great measure to preserve the integrity of the various organs and tissues, yet allowing for each to exert its proper effect upon the others and upon the organism as a whole. The mechanisms by which endothelial permeability is maintained normally or altered in certain pathologic states are thus of the utmost importance, both in health and disease.

Hypoxia ("anoxia") has long been recognized as a potent force for the production of increased permeability, and Landis [1] has beautifully demonstrated this effect and made quantitative determinations upon the extent of such action. He found that 3 minutes of oxygen lack increase filtration through the capillary wall fourfold and that not only did fluid escape in abnormal quantities but that considerable protein also was lost.

Much controversy has arisen over the part played by anoxemia in various types of edema, especially that due to cardiac failure. Current opinion is leaning more and more toward increased capillary pressure as the major factor in this condition, yet, as so aptly stated by Drinker and Yoffey,[2] "Even in well controlled experimental work the effects of venous obstruction, anoxemia, and carbon dioxide increase on capillary permeability cannot be separated from each other with finality; and in clinical conditions these three factors invariably operate together." Similarly, in considering the pathogenesis of surgical shock, the question invariably arises as to the importance of anoxemia and attendant increased capillary permeability. Only a few years ago, the mechanisms by which irreversible shock become established seemed relatively simple. It was thought that following an initial peripherovascular collapse, there developed a relative disproportion between circulating blood and the volume provided by the vascular bed. This resulted in decreased blood flow, stasis, anoxemia, and finally a marked increase in capillary permeability which allowed plasma to leak out into the tissues. A considerable number of experimental studies in the last

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few years have shown conclusively that there is no generalized increased capillary permeability in shock.[3-7]

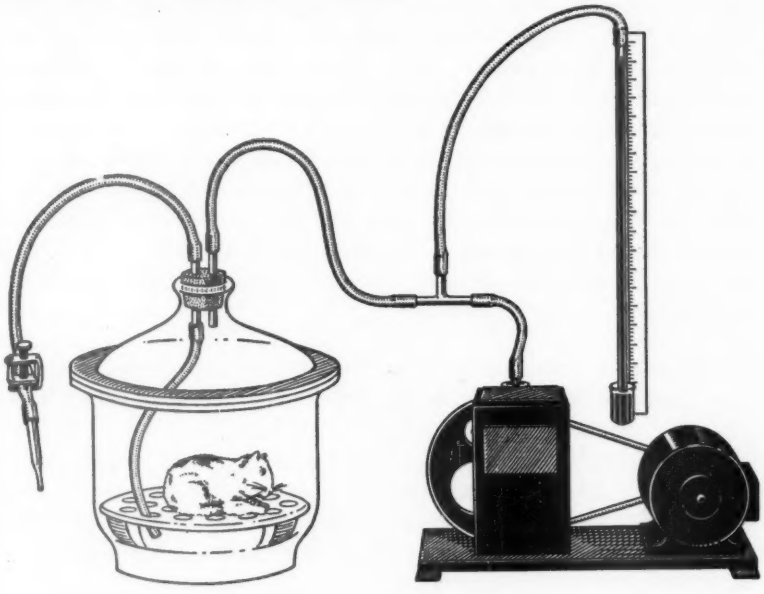
Maurer,[8] in a recent series of experiments, demonstrated that dogs which were subjected to low oxygen tensions responded by an increased production of cervical lymph and that there was an increased passage of protein from the blood capillaries to the lymph. This was accompanied by a corresponding decrease in the concentration of serum protein. McMichael and Morris[9] had previously demonstrated that "breathing mixtures containing percentages of oxygen as low as 9.5 is without effect on the rate of swelling of the human arm." For the most part, however, almost all of the experimental studies which have dealt with capillary permeability and anoxemia have been limited to observations on various localized regions in which a stagnation anoxemia was affected by obstruction to blood flow. Since capillaries of various tissues and regions of the body may behave quite differently, it is apparent that studies on capillary permeability in any local area may not necessarily indicate the reaction of capillaries elsewhere or of the capillary bed as a whole under similar conditions. Neither will the effects of stagnation anoxemia necessarily duplicate the effects of anoxic anoxemia.

With recent developments in the field of aeronautics, exposure to severe degrees of anoxia has become a more common occurrence. Experiments to determine the effects of general anoxemia of this type (anoxic anoxemia) on capillary permeability are therefore all the more important. We are concerned not only with the formation of edema and the pathogenesis of shock, but also with the question whether increased capillary permeability will permit the escape and localization in tissues of bacteria, toxins, antibody globulins, drugs, or other agents which may produce disease or modify inflammatory reaction. We are concerned, in fact, with any alteration of balance among those substances normally maintained in proper proportions by virtue of the semipermeability of capillary endothelium.

Methods and Materials

In order to study capillary permeability, use was made of a phenomenon well known to immunologists, by means of which the escape of antibody globulin through blood capillaries may be determined. This phenomenon is illustrated as follows: If a guinea-pig is passively sensitized to some antigen, an appreciable interval of time (hours) must elapse before that animal becomes susceptible to anaphylactic shock, even though the sensitizing antibodies be administered directly into the blood stream. It is assumed that this so-called *minimum latent*

period of passive anaphylaxis is an expression of the time required for the antibodies to traverse the capillary endothelium and to permeate the tissues so that when the antigen-antibody reaction occurs, vital structures will be damaged and the changes characteristic of anaphylaxis will occur. If anoxic anoxia increases capillary permeability to antibody globulin, the minimum latent period of passive anaphylaxis should be correspondingly shortened.



Text-Figure 1. Decompression chamber.

To produce anoxemia, a relatively simple mechanism was employed, one capable of duplicating rather closely such atmospheric conditions as would be encountered at high altitudes. It consisted of a large pyrex desiccator (8.5 liter capacity), the lid of which contained a two-hole stopper providing two large outlets. One of these outlets was connected with a vacuum pump; the other admitted a rubber tube, one end of which led to the bottom of the chamber, the other end projected to the outside and was fitted with a screw clamp in order that an adequate intake of air could be allowed, and yet the pressure within the chamber could be reduced to, and maintained at, any desired level. A mercury manometer was introduced into the system (Text-Fig. 1). Preliminary studies showed that an atmospheric pressure of 230 mm. Hg, equivalent to 30,000 feet above sea level, was near the limit of toler-

ance for guinea-pigs when maintained at this pressure for a period of 30 minutes. This pressure and exposure time were used throughout all of the experiments. At this pressure, O_2 concentration is 6.4 per cent.[10] The chamber was evacuated at a steady and closely regulated rate during a period of 15 minutes, at which time the desired oxygen concentration ("altitude") was attained.

Young, healthy, adult guinea-pigs were used throughout the experiments, and three were placed in the chamber at one time. In anaphylactic studies, a single source of high titer pooled anti-crystalline-egg-albumin rabbit serum was used. The sensitizing dose of antiserum and the shocking dose of 1 per cent crystallized egg albumin were administered via the jugular vein.

In experiments in which determinations on blood plasma were required, either heparin or a mixture of ammonium and potassium oxalate was used as the anticoagulant. Protein determinations were done by the densimeter method of Barbour and Hamilton.[11] Hemoglobin determinations were made using the Dick-Stevens photo-electric hemoglobinometer.

Experimental Observations

Preliminary experiments showed that, with the particular antiserum used (anti-crystalline-egg-albumin rabbit serum), 0.01 cc., given intravenously, was the minimum lethal sensitizing dose when a shocking dose of 1 cc. of 1 per cent crystallized egg albumin was given 24 hours later; 0.001 cc. of this antiserum would result in a definite but nonfatal anaphylaxis under these conditions. The minimum latent period for fatal anaphylaxis when 0.02 cc. of antiserum was given intravenously was 126 to 135 minutes, although with larger sensitizing doses this minimum latent period could be considerably shortened. In the anaphylactic studies to be described, 0.02 cc. of antiserum (2 times the minimum sensitizing dose) was routinely employed as the sensitizing dose; 1.0 cc. of 1 per cent crystalline egg albumin was the shocking dose. Sensitized control animals were left at normal atmospheric pressure. Treated animals, 5 minutes after sensitization, were placed in the pressure chamber and exposed to an atmospheric pressure equivalent to that found at 30,000 feet ($O_2 = 6.4$ per cent). Table I illustrates the results of such experiments on 20 animals. It is evident that the minimum latent period was not shortened as a result of anoxemia in the 9 treated animals.

Since antibody globulin is one of the larger protein molecules of the plasma, it was considered that some smaller protein molecule, such as serum albumin, might leak through capillaries under these conditions of anoxemia even though globulin did not appear to do so. Conse-

quently, investigations were undertaken utilizing the dye T-1824 (Evans blue). It has been demonstrated [12] that this dye, when mixed with blood or serum, behaves like serum albumin * in so far as its permeability to various membranes is concerned. Five series of experiments on carefully paired (control and treated) guinea-pigs were performed. Paired animals were of almost identical weight and, within each pair, the interval between injection of the dye and the collection of blood samples was the same, ±30 seconds. In order to minimize trauma

TABLE I

Minimum Latent Period for Fatal Anaphylactic Shock in Passively Sensitized Guinea-Pigs

Controls	Interval	Anoxic
	(*minutes*)	
	90	o
	108	oo
	118	o+
o	120	
++	126	o
	128	+
oo	132	
	133	o
++	135	
	136	+
++	137	
+	171	
+	180	

+ indicates fatal anaphylaxis; 0 indicates survival. (Each symbol represents one animal.)

(cardiac puncture) and to protect the animals from preliminary blood loss, which would induce a shift in interstitial fluids and rather marked hemodilution, reliance was placed upon the law of averages to provide relatively equal blood-dye concentration in the control and treated groups following the introduction of a constant dose of T-1824 per kg. of body weight. Determinations of the dye T-1824 were done on plasma. A sufficient amount of dye was injected so that the sample of plasma obtained could be diluted 1:10, in order to minimize blood loss. The dye containing plasma was diluted with beef plasma, and determinations were done with the Evelyn and with the Klett Summerson photo-nephelometers. Samples were centrifuged immediately before reading. All animals received 4 mg. per kg. of body weight of T-1824 intravenously (jugular vein). This material was carefully prepared in a volumetric flask so that 1 cc. of the saline solution contained 1 mg. of the dye. Injections were made with a syringe of tuberculin type, graduated in 0.01 cc.

* With the concentrations of T-1824 used in these experiments, all of the dye would not have been bound to the albumin fraction although the major part of the dye would have been incorporated in the serum albumin.

In one experiment (Table II) only hemoglobin and protein determinations were made. These were made both before and after subjecting the animals to reduced O_2 tension and required less than 1 cc. of blood from each animal. Their purpose was to evaluate any hemoconcentration or dilution brought about by this acute anoxemia which might in turn affect the blood-dye concentration of anoxic animals (see Table II).

TABLE II

The Effect of Anoxic Anoxemia on Disappearance Rate of T-1824, Hemoglobin, and Plasma Proteins

Series	Animals	Average concentration		
		T-1824	Hemoglobin	Protein
		(*mg.* %)	(*gm.* %)	(*gm.* %)
Series 1, paired guinea-pigs: 380–486 gm.; interval, 77′–88′*	Anoxic (3)	7.525		
	Controls (3)	7.465		
Series 2, paired guinea-pigs: 363–446 gm.; interval, 87′–88′*	Anoxic (3)	6.62		
	Controls (3)	5.67		
Series 3, guinea-pigs: 380–454 gm.; interval, 77′–87′†	Before anoxia (6)		14.0	5.39
	After anoxia (6)		12.4	4.51‖
Series 4, paired guinea-pigs: 336–467 gm.; 137′–161′*	Before anoxia (6)‡			5.29
	After anoxia (6)	4.64		4.54
	Controls: 1st dtmn. (6)§			5.30
	Controls: 2nd dtmn. (6)§	4.64		4.70

* Interval of time in minutes elapsing between injection of T-1824 and withdrawal of blood sample.

† Interval of time in minutes elapsing between onset of anoxemia and withdrawal of second blood sample.

‡ Blood sample withdrawn 20 minutes after injection of T-1824.

§ First and second determinations parallel, in time, the withdrawal of blood samples "before" and "after" anoxia.

‖ It may be considered that the variation in hemoglobin in series 3 is a measure of hemodilution resulting from treatment. If protein determination "after anoxia" is corrected on this basis, the decrease in plasma protein is actually 6.1%.

In a fifth experiment, dye, hematocrit, and protein determinations were made in both control and treated animals before and after one-half of the animals were subjected to reduced O_2 tension. Any differences in ratios of blood volume to body weight, the result of hypoxia, could thus be determined and dye and protein concentrations could be corrected for these differences (see Table III).

From these observations it appears that the rate of disappearance of T-1824 is not increased in animals which are subjected to acute anoxic anoxia. There is a suggestion that the rate actually may be decreased as a result of anoxemia. In the one series in which plasma protein determinations were made before and after animals were exposed to decreased O_2 tension and compared with similar determinations in control animals, no significant variation was observed (Table III).

Discussion

On the basis of evidence presented here, one may conclude that acute anoxic anoxia, of a degree which approximates or slightly exceeds the minimal lethal range for human beings, does not produce a detectable increase in capillary permeability to plasma proteins in guinea-pigs, although three entirely different methods were employed to determine such an effect. These findings correlate well with similar observations

Table III

The Effect of Anoxic Anoxemia on Disappearance Rate of T-1824, Plasma Protein, and Hematocrit Determination

Series	Animals	Average concentration		
		T-1824	Protein	Hematocrit
		(mg. %)	(gm. %)	(%)
Series 5, paired guinea-pigs: 575–740 gm.; interval,* 112′–114′	Before anoxia (5)†	9.39	4.55	41.1
	After anoxia (5)	6.10	3.81	36.1
	Controls: 1st dtmn. (5)‡	10.03	4.85	42.5
	Controls: 2nd dtmn. (5)‡	6.47	4.31	38.1

* Interval of time elapsing between injection of T-1824 and withdrawal of second blood sample.

† Blood sample withdrawn 20 minutes after injection of T-1824.

‡ First and second determinations parallel, in time, the withdrawal of blood samples "before" and "after" anoxemia. It may be considered that the variation in hematocrit reading is a measure of hemodilution resulting from treatment. When the second determinations of T-1824 and of protein are corrected on this basis, the variation is:

Animals	T-1824	Protein
Anoxic (5)	−26.1%	−4.6%
Controls (5)	−28.1%	−0.8%

on the state of capillary permeability in conditions of surgical shock.[3-7] In the careful experiments of Landis,[1] and others, which have shown an increased regional capillary permeability from stagnation anoxemia, it seems probable that alteration in carbon dioxide was the major factor in producing this effect.

Summary and Conclusion

1. The minimum latent period for anaphylactic shock in guinea-pigs following passive sensitization is presumed to be an indication of the time necessary for antibodies to escape from the blood stream into the tissues. This minimum latent period was not shortened by acute anoxic anoxemia brought about by subjecting passively sensitized guinea-pigs to low oxygen tensions. Therefore, anoxemia, under these conditions, does not facilitate the passage of antibody globulin through vascular endothelium.

2. Studies on the rate of disappearance of T-1824 from the blood stream indicate that acute anoxia does not increase the normal rate of disappearance of this dye. This dye, when mixed with blood or serum, behaves like serum albumin in so far as its permeability to various membranes is concerned. Therefore, anoxemia, under the conditions described, does not facilitate the passage of serum albumin through vascular endothelium.

3. Significant alterations in the quantity of plasma protein following acute anoxic anoxia were not observed.

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THE PATHOLOGY OF MALIGNANT CATARRHAL FEVER (BOVINE EPITHELIOSIS)

WITH SPECIAL REFERENCE TO CYTOPLASMIC INCLUSIONS *

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Malignant catarrhal fever is an infectious, noncontagious, sporadic, highly fatal disease of cattle. Since the successful transmission of the disease by blood inoculation reported by Goetze and Liess,[1] the etiologic agent has been thought to be a filterable virus. However, their filtration experiments and inoculations with blood plasma gave only negative results. Others were able to produce the disease in cattle by intravenous and subcutaneous inoculation of whole blood, but filtrates of such blood were not infective. No microorganisms could be demonstrated in the whole blood used for inoculation.[2]

In their studies in South Africa, du Toit and Alexander [3] reported nonfilterability of the virus and explained this characteristic by suggesting that the virus was closely associated with erythrocytes.

Since cell inclusions are found in many virus diseases, a detailed search was made for their presence in malignant catarrhal fever. Of 18 cases of this disease occurring in one community, a detailed study of the pathologic changes was made in 3. This search revealed inclusion bodies in numerous tissues in all cases. The finding of these cytoplasmic cell inclusions in these cases should help establish the viral causation. Demonstration of inclusions may become a laboratory procedure which will aid in a positive diagnosis of this disease.

CLINICAL DATA

The history and symptoms of all cases were similar. Animals of both sexes and ages from 10-months-old calves to mature animals were affected. The course varied from 5 to 14 days, and only 2 of 18 affected animals recovered. All cases occurred on different farms except for 4 which were on two farms on each of which 2 animals were affected.

Excessive lacrimation, congestion of the sclera and conjunctiva, extreme dejection, dysphagia, dyspnea, and a temperature of 106° to 108°F. were noted on the first day. Later, cloudiness of the cornea occurred, as well as excessive salivation, with papule and vesicle formation on the muzzle, skin, and buccal, labial, gingival, and pharyngeal mucosa. The serous inflammation of the oral, nasal, and vaginal mu-

* Received for publication, October 8, 1946.

cosa changed to sero-catarrhal, then catarrhal or croupous, and finally there were patches of epithelial excoriation from the muzzle and from the labial, pharyngeal, and gingival regions. A fetid odor was emitted from the mouth and nostrils due to necrosis of the muzzle and mucous membranes.

The vesicles in the skin ruptured, leaving moist areas, followed by drying of the serous fluid and epithelium, forming parchment-like crusts in the hair. Near the termination of the disease, the corium of the horns became detached from the corneal processes when bumped or grasped.

Gross Findings

The anatomic diagnoses included: papular and vesicular dermatitis, especially pronounced on the withers, forearms, scrotum, and teats; easy detachment of the corium of the horns from the cornual processes; serous, sero-hemorrhagic, croupous and diphtheritic stomatitis, pharyngitis, rhinitis, and vaginitis; swollen submaxillary and anterior cervical lymph glands; and conjunctivitis. The corneas were opaque and more than twice as thick as is normal.

Histopathologic Findings

Tissues were fixed in 10 per cent formalin, embedded in paraffin, cut at 4 μ, and stained with hematoxylin and eosin or Shorr's III.[4] The tissues were taken from animals *in extremis*.

The nasal mucosa showed extensive desquamation of the epithelium with congestion and hemorrhage in the submucosa. Cytoplasmic, homogenous, acidophilic, and basophilic inclusions were observed in the epithelial cells.

The pharyngeal mucosa and tongue showed numerous vesicles and submucosal hemorrhage. Karyorrhexis and pyknosis of the nuclei of the epithelium were present. In all tissues there was practically no leukocytic response to the damage incurred, only a mild eosinophilic leukocytic infiltration of the submucosa being observed.

The opacity of the cornea noted grossly was due to edema in the substantia propria. The connective tissue lamellae of the substantia propria were separated by wide spaces filled with edema fluid, resulting in a cornea measuring from 2.5 mm. (center) to 4.6 mm. (periphery) in thickness. Normal corneas taken from animals after slaughter measured 1.0 mm. to 1.8 mm. in thickness.

Sections of the brain showed petechial hemorrhages of the cerebrum and cerebellum. The Purkinje cells were degenerated and an occasional acidophilic cytoplasmic inclusion was observed in these cells. Perivascular glial accumulations were seen in the white matter of the cerebellum.

Blood smears showed marked erythrocytic anisocytosis.

Smear preparations were made of scrapings from the conjunctival, nasal, oral, and pharyngeal mucosa to determine the presence of inclusion bodies. When stained with Shorr's III [4] or hematoxylin and eosin, three general types of inclusions were observed in the cytoplasm of the epithelial cells. Most numerous were cells showing diffuse, granular, basophilic, cytoplasmic inclusions which measured 0.25 to 0.50 μ in diameter. The number of inclusions per cell varied from only a few, to some instances in which the cytoplasm was packed with these bodies. The second type of inclusion observed consisted of clustered, granular, basophilic cytoplasmic bodies measuring from 1.75 to 5.25 μ in diameter. The granules composing these bodies were of the same size as those observed scattered diffusely throughout the cells described above. The number per cell varied from one to fifteen. Often the cells possessing the clustered inclusions also contained several of the scattered granules. The third type of inclusion consisted of sharply defined homogeneous bodies usually staining acidophilic, although some were neutrophilic. These measured from 2.0 to 5.25 μ in diameter, the majority being 3.5 μ. According to the work of Lucas and Riser [5] on inclusions of panleukopenia (infectious enteritis) of cats, these types may represent various stages in the formation of the mature homogeneous inclusion, starting with diffuse granular bodies.

Shorr's III stain [4] gave the most satisfactory results, offering a sharper distinction to the inclusion. Hematoxylin and eosin stain also gave fairly satisfactory results. Giemsa's stain gave poor differentiation between cytoplasm and inclusions. Seller's [6] stain failed to stain the inclusions. Azur II and eosin failed to give cellular detail.

For critical study of cellular detail, a binocular Zeiss microscope was employed using the apochromatic 120 ×, 1.30 n.a. objective and 10 × compensating eyepieces. Additional magnification of 1.5 × was given by the Zeiss binocular. The 1.2 condenser was always focused as carefully as the objective. A Bausch and Lomb gas-filled projection mazda lamp was the source of illumination.

Summary and Conclusions

Malignant catarrhal fever is characterized primarily by a serous or sero-catarrhal inflammation largely involving the epithelial tissues, followed by varying degrees of catarrhal, croupous, and diphtheritic inflammation of the mucous membranes. It is accompanied by neuronal degeneration and hemorrhage in the brain.

Cytoplasmic inclusion bodies are present in the epithelial cells of mucous membranes. Three types were observed: diffuse granular, clustered granular, and homogeneous.

The finding of cytoplasmic inclusions in the epithelial cells adds to the evidence supporting viral causation.

The demonstration of these cytoplasmic inclusions may be a laboratory procedure which will aid in differentiating malignant catarrhal fever from vesicular stomatitis, rinderpest, acute infectious aphtha, and aphthous stomatitis.

Since the lesions involve epithelial structures and the inflammation is primarily of a serous or sero-catarrhal type, it is suggested that bovine epitheliosis is a more appropriate and descriptive name than malignant catarrhal fever.

We wish to express our appreciation to Dr. L. C. Prushing of Mt. Vernon, Ohio, for assistance in obtaining clinical material.

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DESCRIPTION OF PLATE

PLATE 132

FIG. 1. Epithelium, nasal mucosa. Neutrophilic homogeneous cytoplasmic inclusions. Hematoxylin and eosin stain. × 1800.

FIG. 2. Epithelium, conjunctival mucosa. Acidophilic homogeneous cytoplasmic inclusions. Hematoxylin and eosin stain. × 1800.

FIG. 3. Epithelium, pharyngeal mucosa. Clustered granular and diffuse granular cytoplasmic inclusions. Shorr's III stain.[4] × 1800.

FIG. 4. Epithelium, oral mucosa. Homogeneous and granular inclusions. Shorr's III stain.[4]

FIG. 5. Pharyngeal mucosa. Vesicle. Hematoxylin and eosin stain. × 150.

FIG. 6. Nasal mucosa. Catarrhal rhinitis. Hematoxylin and eosin stain. × 150.


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DEVELOPMENT OF THE INFLAMMATORY LESIONS AND OF RICKETTSIAE OF MURINE TYPHUS IN THE LUNGS OF RATS *

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Although, during recent years, important progress has been made in our knowledge of the typhus virus, the mode of multiplication of rickettsiae remains obscure. Various theories have been proposed. One of the most common is the ultravirus theory advocated by Chandler [1] and others, which assumes that the smallest visible forms of the pleomorphic typhus virus are in continuity with others, smaller still, invisible with the ordinary microscope. It finds support in the difficulty or even impossibility of finding any rickettsiae in the earliest typhus lesions developing in certain animals. On the other hand, Begg, Fulton, and van den Ende [2] suggested the existence of a developmental cycle, somewhat similar to that of psittacosis virus.[3] In this cycle, especially during the period of adaptation to a new host, the virus passes from the earliest stage of "homogeneous inclusion bodies" to the stage of "morulae," which mature progressively to reach the adult stage when the limiting membrane is disrupted and the rickettsial masses are discharged. To elucidate the way in which rickettsiae multiply, a series of experiments has been carried out, with the results here recorded.

METHOD

Rats were infected according to Castañeda's method.[4] One cc. of a suspension, prepared by grinding the lung of a mouse infected with murine typhus in 4 cc. of 10 per cent horse serum broth and storing at —76°C. in a mixture of alcohol and solid carbon dioxide, was introduced intranasally. One animal died 3 minutes after infection, the others were killed with chloroform after 1, 3, 5, 7, 9, 24, 33, 48, and 55 hours; between the 72nd and the 96th hours most of the animals died, the survivors being killed with chloroform. The rickettsiae were stained with methyl violet and metanil yellow [5]; the sections for examination of the inflammatory lesions, with hematoxylin and eosin.

DEVELOPMENT OF LESIONS IN THE LUNG

Macroscopic Appearance. Three minutes after infection, the lungs were congested, particularly the upper and central parts around the hilum. As time passed, the congestion became more marked and progressively involved the entire lung, being followed, after 48 hours, by

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foci of consolidation which increased in size and became confluent, so that in animals dying between the third and fourth days large areas of grayish red to deep red consolidation were found. Moreover, exudate, which is a marked feature in mice, was never really abundant except sometimes in the pyramidal lobe at the terminal stage of the inflammatory process. In the pleural cavity exudate has never been observed.

Microscopic Appearance. Three minutes after infection, the capillaries of the lung were dilated and numerous alveoli were filled with the inoculum. In lungs fixed 1 hour later, polymorphonuclear leukocytes were present in increased numbers in the capillaries and a few were seen in the cellular interstices of the alveolar septa. Some of the alveolar cells were slightly swollen and a few were desquamated into the alveolar spaces. After 3 hours, the swelling of the cells increased, more cells were involved, and many of the alveolar spaces were narrowed. Exudation of fluid into the alveoli began at this early stage. Five hours after infection, groups of alveoli lined with enlarged alveolar cells and surrounded by almost normal alveoli were seen. In these foci the alveolar spaces contained desquamated alveolar cells in greater numbers and also a few polymorphonuclear leukocytes. After 7 hours, cuffs of small lymphocytes mixed with a few polymorphonuclear leukocytes appeared around the blood vessels of the bronchioles. After 9 hours, the alveolar spaces in the focal areas were filled with swollen and desquamated alveolar cells, and inflammatory edema was abundant. Twenty-four hours after infection, plasmacytes appeared around the capillaries; the foci had increased in size and contained a greater number of obliterated alveoli. After 33 hours, and still more after 48 hours, the foci of consolidation merged into one another, more parenchyma being thus progressively involved in the inflammatory process, which reached its full extent and intensity 72 hours after infection. At this stage, large portions of the lung were consolidated, the alveolar spaces being filled with swollen desquamated alveolar cells, leukocytes, and inflammatory edema. The remaining portions of lung were congested and might contain a few foci of consolidation. All over the lung, but particularly in the consolidated portions, numerous polymorphonuclear leukocytes, lymphocytes, and plasmacytes were seen, singly or in groups. Thick cuffs of small monocytes surrounded the bronchioles and blood vessels, together with variable numbers of the aforementioned cells. The bronchial epithelium showed some swelling, particularly at the terminal stages, but a massive desquamation never occurred. The bronchioles were filled with leukocytes and desquamated alveolar cells, among which a few bronchial epithelial cells and cellular

débris were found. The pleura over nonconsolidated areas showed no change, but over consolidated parts the mesothelial cells were enlarged and often vacuolated; sometimes they degenerated and disappeared.

Development of the Rickettsiae

Three minutes after infection, rickettsiae were found in the inoculum filling the alveoli and scattered among the cells of the interalveolar septa (Fig. 1). In all animals examined, they were more numerous in the upper and central than in the lower portions of the lungs. Dot forms and coccobacillary types predominated, diplococci and rod forms were scanty. At that stage the organisms stained with great difficulty. After 1 hour, fairly numerous rickettsiae could be seen inside the alveolar cells or on their surface (Fig. 2). As time passed they appeared in increasing numbers in the alveolar cells and in the epithelial cells of the bronchial tree, becoming gradually less numerous, though never absent from the alveolar spaces. At this early stage the rickettsiae began to undergo important morphologic changes, dots becoming less numerous while coccobacilli and rods increased in number. The morphologic changes were visible after 3 hours (Fig. 3), but they became conspicuous after 5 hours. At this stage small, irregularly shaped, dense clumps appeared in which the organisms, unlike those composing the loose groups probably of purely mechanical origin and which were seen 3 hours after infection (Fig. 3), could not be seen along their whole length (Fig. 4). These clumps were situated in the interalveolar septa, inside the alveolar cells or on their surfaces. At this stage, diplobacillary forms of rickettsiae appeared, twice as large as a coccobacillus (Fig. 4 insert), and also rod forms which were considered to be of the adult type since their length and staining properties were similar to those which are commonly found at the terminal stage. Seven and 9 hours after infection, the adult rods were more numerous and the clumps increased both in number and size (Figs. 5 and 6). Some of the clumps, fairly numerous but still irregular in shape after 24 hours (Fig. 7), acquired, after 33 hours, a rounded shape and a granular structure and formed small granular bodies, while those remaining retained their irregular shape (Fig. 8). At 48 hours, the differentiation of the clumps into two types of unlike structure, the granular bodies and the irregularly shaped aggregates, became conspicuous (Fig. 9). The granular bodies were characterized by a rounded form, sharp edges, and dense structure; the irregularly shaped aggregates were ill defined and usually of less dense structure than the granular bodies. From both, individual rickettsiae grew out into neighboring cellular interstices and alveolar spaces. Fifty-five hours after infection, granular bodies and

aggregates had increased considerably in size (Fig. 10), and after 72 hours they had reached their full size (Fig. 11). During this period, single rickettsiae, grown out in large numbers from the infected cells, infiltrated tissue interstices and alveoli filled with inflammatory edema and numerous organisms. There was, however, considerable variation in the number of rickettsiae in individual animals.

Rickettsiae do not necessarily develop in clumps. From the early stages many alveolar cells contained individual rickettsiae which multiplied actively, so that their cytoplasm was first dotted with individual organisms and later filled with diffuse masses of rickettsiae from which individual organisms grew out into the neighboring alveolar spaces (Fig. 32). This diffuse development could be observed only in certain alveolar cells, but it was common in the bronchial epithelial cells. It was difficult to decide when the multiplication of rickettsiae began in these cells, since in the early stages they might increase in number within the cells either by multiplication or by the inclusion of new organisms.

In animals killed 72 to 96 hours after infection, extracellular and intracellular rickettsiae were very numerous, being more abundant in the consolidated (Fig. 12) than in the nonconsolidated (Fig. 13) portions of the lungs. Extracellular rickettsiae could be seen particularly in the tissue interstices and in the inflammatory edema filling the alveoli, either singly (Fig. 14) or in clumps (Fig. 15). Stained purple, the organisms stood out sharply from the yellow-stained exudate, and all forms mentioned above could readily be made out, with numerical predominance of the longer forms over the dots (Fig. 14). Quite often, single rickettsiae and clumps coexisted within the same alveolar space, with no sharp limits between them (Fig. 15). In some alveoli which had become confluent through rupture of the interalveolar walls, very large and fairly well defined rickettsial masses were seen which were about twenty times the size of an alveolar cell. These masses were similar to those which have been described in mice, but most of them were more voluminous.[5] Quite often, rickettsiae were seen at the peripheries of these masses, singly or in clumps, scattered in the surrounding inflammatory edema. The intracellular rickettsiae, as in mice, were particularly numerous in the alveolar cells. Almost all of these cells were infected in the terminal stages, but the number and arrangement of the organisms within them varied greatly. Some cells contained only a few organisms (Figs. 16 and 17); some were partly (Figs. 18, 19, 20, 23, and 26) and others entirely (Figs. 21, 22, and 27) filled with diffuse rickettsial masses. Besides these cells, others could be seen which contained in their cytoplasm one (Fig. 17) or several (Fig. 18) aggregates. If a cell contained several aggregates, they might be arranged at the

periphery (Fig. 18), or around the nucleus which might be surrounded by rickettsiae. Quite often, aggregates could be seen on the surface of the cytoplasm of the harboring cells, protruding into the cellular interstices (Figs. 24 and 25). In some cells aggregates coexisted with single rickettsiae (Fig. 17). Numerous cells contained granular structures similar to those described in mice.[5] In rats, too, the granular bodies varied greatly in size, shape, and structure. Besides granular bodies not larger than the smallest blood-platelet, others could be seen as large as those shown in Figures 28 and 29, distending and entirely filling the cytoplasm of the harboring cells. Usually round or oval, they might be fusiform, pyriform, triangular, beaded, or otherwise irregular. Often they had one or more rounded, lateral projections (Fig. 29). Although most of them were dense in structure, in some granular bodies the organisms were arranged less closely.

No strict separation could be made among the irregularly shaped aggregates, the diffuse rickettsial masses filling the cells, and the granular structures; intermediate forms were readily found (Fig. 27). Frequently, aggregates and granular bodies coexisted in the same cells (Fig. 30). Sometimes the granular bodies were studded with clear spots exhibiting the staining affinities of cytoplasm (Fig. 29). In no case was any limiting membrane seen. Most of the bronchial epithelial cells were stuffed with diffuse masses of rickettsiae. It seems worth noting that these cells never developed characteristic granular bodies, and that no cells containing only a few rickettsiae could be made out at the terminal stages; either they were packed with organisms or free of them. In and among the desquamated cells and cellular débris filling the bronchioles, innumerable rickettsiae could be seen, sometimes in clumps but usually single and normal in appearance. Such granular bodies as could be seen in certain desquamated alveolar cells showed no sign of alteration in their structure. In rats, as in mice, the polymorphonuclear leukocytes contained comparatively few rickettsiae, usually coccobacilli and rods, normal in appearance. Endothelial cells of the capillaries rarely showed rickettsiae. If they were infected, their cytoplasm was filled with uniform masses of rickettsiae or with small but numerous granular bodies. The infected cells were swollen and projected into the lumina of the capillaries.

Discussion

Nature of the Inflammatory Process

This study shows the primarily focal character of rickettsial bronchopneumonia in rats, which differs from that found in mice only in its lesser intensity and extent, while the inflammatory edema also is less abundant. The cellular changes, however, show a noticeable difference.

The swelling of the alveolar cells is always very marked and desquamation of the swollen cells resulting in obliteration of numerous alveolar spaces is a prominent feature. The abundant desquamation and elimination of infected alveolar cells which, mixed with numerous polymorphonuclear leukocytes containing phagocytized rickettsiae and a great number of single rickettsiae, are found filling most of the bronchioles, can be regarded as a strong cellular reaction of the rat against rickettsial infection. This idea is strengthened by the fact that polymorphonuclear leukocytes appear as early as 1 hour after infection. Although no bacteriologic examinations have been carried out to eliminate the possibility of coexistent bacterial infection, the reaction seems to be directed against the rickettsiae since no bacteria have been found in the sections.

Development of Rickettsiae

Four phases can be distinguished in the development of rickettsiae in the lungs of rats: (1) they appear within the cells, (2) they undergo morphologic changes, (3) they multiply, (4) they grow out from the infected cells. Whatever may be the mechanism by which rickettsiae become intracellular, it is certain that very soon after the introduction of the rickettsial suspension into the lung many alveolar cells become infected, and that the number of intracellular organisms increases progressively, while extracellular organisms become less numerous. Parallel with this, extracellular as well as intracellular rickettsiae undergo morphologic changes, the dot forms becoming less numerous, while the coccobacilli and rods increase in number. The most striking morphologic change is the appearance, 5 hours after infection, of adult rods and diplobacilli. Their appearance is associated with that of small intracellular clumps which are considered to be the earliest colonies of rickettsiae. It is therefore difficult to escape the conclusion that morphologic changes of the rickettsiae are related to their multiplication. The colonies are thus assumed to originate from the multiplication by transverse fission of single rods or diplobacilli, the new organisms remaining together, multiplying in their turn, and thus increasing the size of the colonies. This view is supported by the fact that rods and diplobacilli very often are found in the neighborhood of early colonies (Fig. 5). It is impossible to say whether the dot forms have to go through the stages of coccobacilli, adult rods, and diplobacilli before multiplying, or whether they can multiply directly. When the clumps have reached a certain size, they differentiate into granular bodies and irregularly shaped aggregates; in later stages, individual rickettsiae grow out of them in increasing numbers until, in the terminal stages, all cellular interstices are invaded.

No stage has been encountered in which rickettsiae are absent. Therefore, it appears unnecessary to postulate the existence of an ultramicroscopic phase.

Extracellular Growth of Rickettsiae

Once set free, rickettsiae may gain entrance into other cells and grow within them, or remain extracellular and multiply. The extracellular growth is demonstrated by the development of numerous extracellular clumps of organisms such as are shown in Figure 15. These clumps are considered to originate from the multiplication of single extracellular rickettsiae and, therefore, to be colonies of rickettsiae similar to those described inside cells. In support of that view it can be put forward that the size of these clumps is not fixed but increases progressively. In Figure 15, the whole process of their development can be followed, from single rickettsiae through clumps consisting of only a few organisms, as are seen on the right side of the figure, to structures as large as those shown on its left side. Increasing further in size, the clumps merge into one another, giving rise, in some animals, to the voluminous rickettsial masses described previously.

The study of sections of lungs taken at various intervals after infection shows that the multiplication of extracellular rickettsiae is seen only at the terminal stages of rickettsial bronchopneumonia. It shows that extracellular rickettsiae, although always present in the infected lungs, are not increasing but diminishing in number up to the 48th hour, when, as has already been mentioned, individual rickettsiae begin to grow out from the infected cells. It is conjectured that the extracellular growth of rickettsiae, universally believed to develop only inside living cells, becomes possible in the lungs of rats and mice[5] at a moment when the substances indispensable to their multiplication and contained in the cells are released from injured cells.

The interpretation of the extracellular clumps as agglutinated rickettsiae can hardly be put in accordance with what is known about the time of development of the agglutinins. No animal whose lung has been used for this study has survived up to the 96th hour after infection, and the antibodies are known to appear in appreciable amount only about 1 week after the contact between the microbes and the host.

Granular Structures in the Life Cycle of the Typhus Virus

Since rickettsiae grow in the cells of the lungs of rats as granular bodies and aggregates or diffusely, the granular structures, which seem to correspond to the "morulae" described by Begg, Fulton, and van

den Ende [2] and also to the "intracellular globular masses" found by Wolbach, Todd, and Palfrey [6] in human typhus, cannot be considered as a specific and indispensable phase in the development of the typhus virus. This view is supported by the fact that granular bodies have never been seen in the yolk-sac membrane, in the tunicae of guinea-pigs so far examined in histologic sections, or in the epithelial cells of the gut of the louse, their natural medium.[6] Moreover, in the lungs of mice and rats granular bodies are never seen in bronchial epithelial cells in which rickettsiae are as numerous as in alveolar cells. In addition, the shape and structure of the granular bodies make it unlikely, in accordance with the observation of Begg and associates, that they play the same rôle in the development of the typhus virus as the "morulae" in the development of the psittacosis virus. Although fairly characteristic, the shape of the granular bodies is variable. Transitional stages exist between granular bodies and diffusely growing rickettsial masses which often merge into a fully characteristic granular body (Fig. 31). Granular bodies are not cystic structures filled with rickettsiae because they have no limiting membrane and also because they are not composed of masses of loose rickettsiae but, as shown in Figure 29, of cytoplasm stuffed with organisms. Finally, no disrupting or disrupted granular body has ever been seen in the evolution of typhus virus in the lungs of several hundred mice and rats so far examined. For these reasons the granular bodies cannot be considered as specific structures representing an obligatory phase in the development of rickettsiae, and the suggestion of the existence of a development cycle as proposed by Begg, Fulton, and van den Ende [2] cannot be accepted.

The granular bodies are regarded merely as colonies of rickettsiae. It has been demonstrated that they develop from the intracellular clumps observed 5 hours after infection, which are the earliest colonies or growing centers of rickettsiae. The fact that granular bodies develop in alveolar cells but are never seen in the cells of yolk-sac membranes, in the cells of the louse gut, or in bronchial epithelial cells, shows that the main factor in their development is the nature of the cells, the strain of rickettsiae used influencing only their number and size. Experiment shows, indeed, that every strain, whether murine or epidemic, gives rise to the development of granular bodies in the alveolar cells, but that the number and size of these structures vary with the strain.

Summary

1. In rats, intranasal infection with typhus rickettsiae results in a bronchopneumonia strongly focal in character. The foci merge into one another and the inflammatory process thus extends progressively. In

the terminal stages large portions of lung are consolidated, the upper and central parts being usually the more severely involved, but not as regularly as in mice. The consolidation is neither so intense nor so extensive as in mice. Exudate is scanty but marked swelling and desquamation of the alveolar cells are prominent features.

2. It is considered that the desquamation and massive elimination of infected alveolar cells and individual rickettsiae are the expression of a strong, though not specific, reaction of the rat lung to typhus virus.

3. Rickettsiae are found in every stage of their development in the lung. Therefore, the existence of invisible forms of the typhus virus seems improbable.

4. In the rat lung, rickettsiae are progressively engulfed by the cells and at the same time undergo characteristic morphologic changes. Then they multiply by transverse fission like bacteria, develop granular bodies and irregularly shaped aggregates, or grow diffusely within the cells. Finally, individual rickettsiae grow out from the infected cells. These organisms may be taken up by other cells or may develop extracellularly, giving rise to extracellular clumps and voluminous rickettsial masses. The extracellular growth of rickettsiae is thus demonstrated.

5. The granular bodies are colonies or growing centers of rickettsiae. There is no evidence of their being a specific phase in a developmental cycle of the typhus virus.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 133

All rickettsiae were stained with methyl violet and metanil yellow. × 1250.

FIG. 1. Three minutes after infection. Minute dots and coccobacilli among the cells of a septum (top), and in the inoculum inside an alveolus (bottom).

FIG. 2. One hour after infection. Fine dots and tiny diplococci in the alveolar cells of a septum.

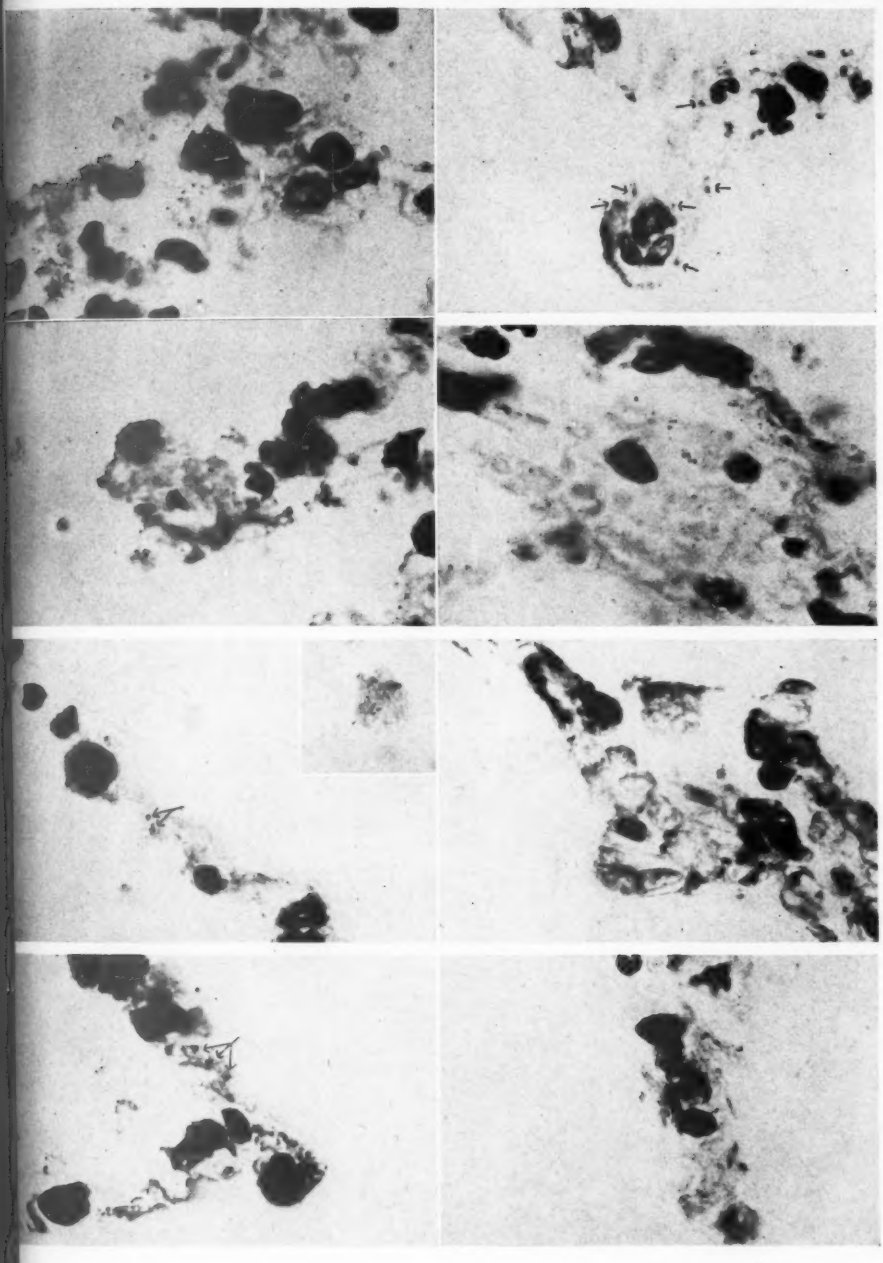
FIG. 3. Three hours after infection. Fine dots, coccobacilli, and rods, singly or in small groups.

FIG. 4. Five hours after infection. Two small clumps in the cells of a septum. Insert shows two diplobacilli in exudate.

FIG. 5. Seven hours after infection. Two clumps (above) and a single adult rod (below) in the cells of a septum.

FIG. 6. Nine hours after infection. Further development of the clumps.

FIG. 7. Twenty-four hours after infection. Clumps increased in number and size located in a septum.



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Rickettsiae of Murine Typhus

PLATE 134

FIG. 8. Thirty-three hours after infection. Earliest granular bodies and a few clumps.

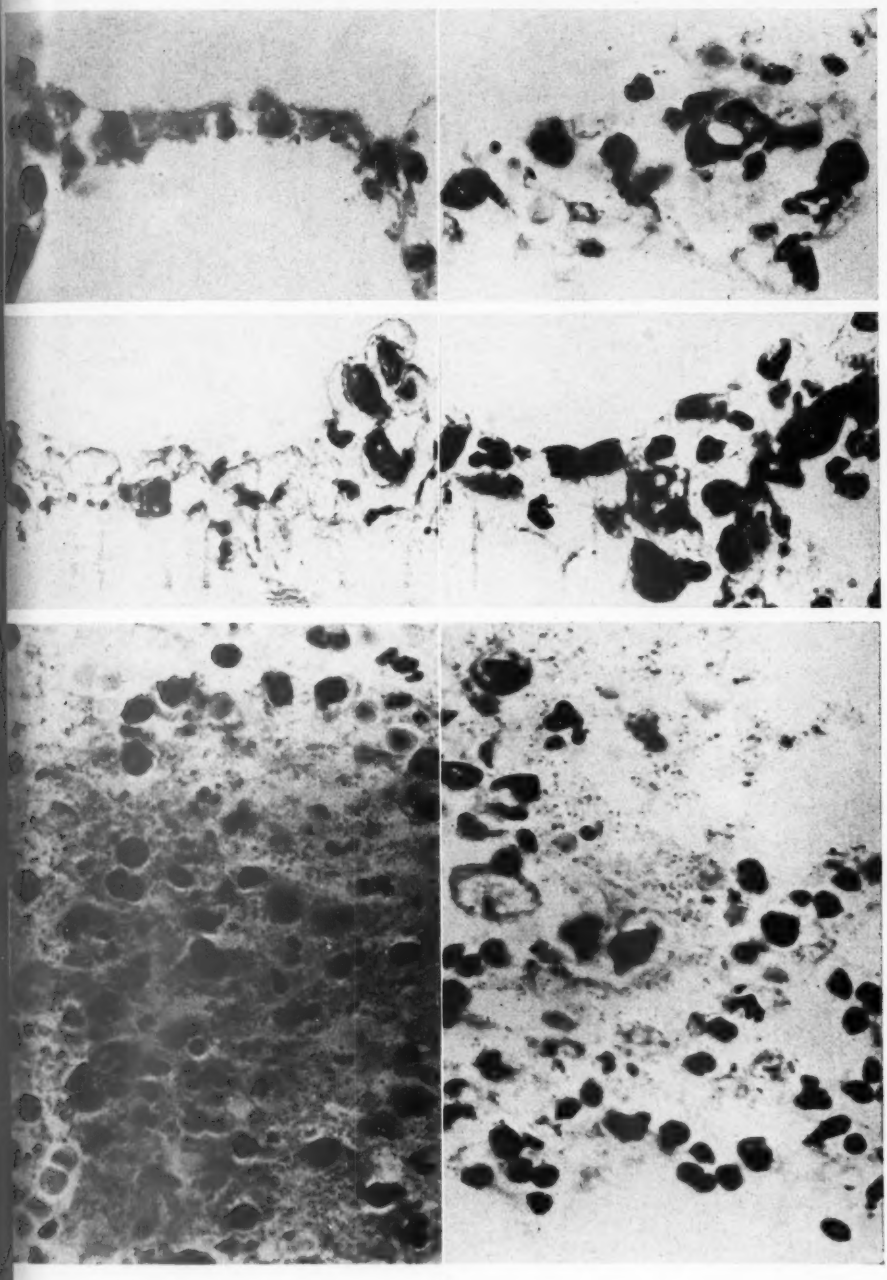
FIG. 9. Forty-eight hours after infection. A few granular bodies and several aggregates in a slightly thickened septum.

FIG. 10. Fifty-five hours after infection. Numerous, fairly large granular bodies and irregularly shaped aggregates within a thickened septum.

FIG. 11. Seventy-two hours after infection. Numerous full-grown, well defined, fairly regular granular bodies and irregular dense aggregates.

FIG. 12. Rat, dead 4 days after infection. Very numerous intracellular and extracellular rickettsiae in the consolidated parts of the lung.

FIG. 13. From the same animal as Figure 12. Fairly numerous rickettsiae among and in the alveolar cells of the slightly thickened septa in the nonconsolidated part of the lung.



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Nyka Rickettsiae of Murine Typhus

FIG. 14. From the same animal as Figures 12 and 13. Very numerous rickettsiae in the exudate filling an alveolus. Numerical preponderance of coccobacillary and rod types over the dot forms.

FIG. 15. From the same animal as Figures 12 to 14. Clumps of rickettsiae and single organisms in an alveolus filled with exudate.

(Figures 16 to 32 were taken from animals which died or were killed 3 or 4 days after infection.)

FIG. 16. Swollen alveolar cell with a few coccobacilli and rods in the cytoplasm.

FIG. 17. Swollen alveolar cell with a few dots and coccobacilli and a small, irregular, fairly dense aggregate in the peripheral part of the cytoplasm.

FIG. 18. Alveolar cell containing in the peripheral part of the cytoplasm some more or less dense, irregular aggregates of various sizes.

FIG. 19. Crescent-shaped aggregate of rickettsiae arranged around the nucleus of an alveolar cell.

FIGS. 20 and 21. Alveolar cells filled partly (Fig. 20) or entirely (Fig. 21) with diffusely growing rickettsiae.

FIG. 22. Alveolar cell with its cytoplasm filled with diffuse masses of rickettsiae and divided into a central and peripheral part by a clear line running parallel to the edge of the nucleus.

FIG. 23. Ring-shaped alveolar cell infected with rickettsiae arranged in the peripheral part of the cytoplasm.

FIG. 24. Irregular, fairly well defined, dense aggregate growing on the surface of a swollen alveolar cell.

FIG. 25. Alveolar cell with a few single rickettsiae inside the cytoplasm and a well defined, irregular, small aggregate on the surface of the cytoplasm.

FIG. 26. Alveolar cell with diffusely growing rickettsiae arranged in the peripheral part of the cytoplasm.

FIG. 27. Alveolar cell with cytoplasm stuffed with rickettsiae, remarkable for sharp outlines and regular shape, resembling a granular body.

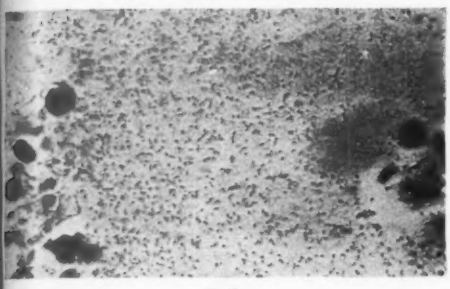
FIG. 28. Alveolar cell with a handle-shaped nucleus (left) and cytoplasm transformed into a well defined and compact granular body.

FIG. 29. Voluminous granular body with a lateral protuberance studded with islets of rickettsiae-free cytoplasm.

FIG. 30. Alveolar cell containing a pyriform granular body and an irregular aggregate of rickettsiae.

FIG. 31. Alveolar cell filled with diffuse, homogeneous masses of rickettsiae merging into an oval granular body.

FIG. 32. Voluminous alveolar cell with cytoplasm filled with homogeneous, diffuse masses of rickettsiae from which single organisms grow out into the surrounding exudate.



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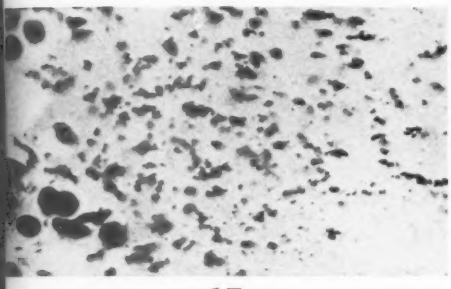

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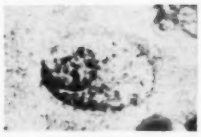

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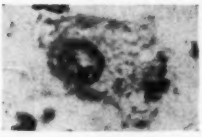

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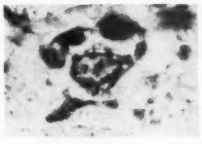

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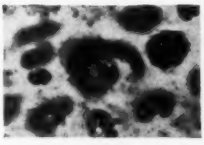

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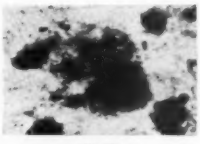

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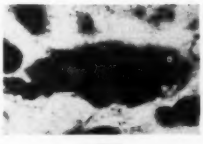

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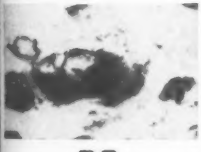

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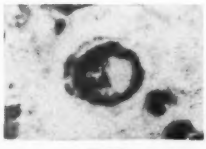

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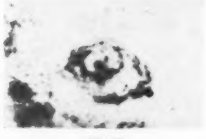

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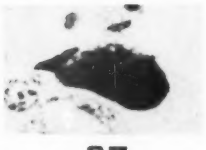

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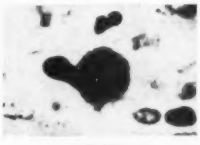

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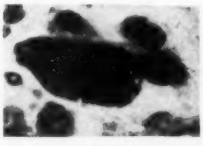

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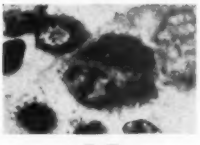

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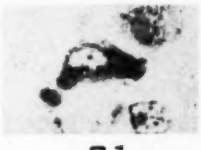

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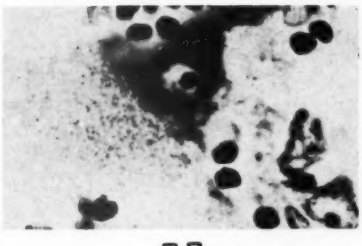

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Nyka

Rickettsiae of Murine Typhus

FORTY-FOURTH ANNUAL MEETING

OF THE

AMERICAN ASSOCIATION OF PATHOLOGISTS

AND BACTERIOLOGISTS

CHICAGO

MAY SIXTEENTH AND SEVENTEENTH, 1947

THE AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

Forty-Fourth Annual Meeting, University of Chicago, Chicago, Illinois

May Sixteenth and Seventeenth, 1947

PRESIDENT FORBUS IN THE CHAIR

BUSINESS MEETING

May Sixteenth, 1947

For the Council, the Secretary announced the following actions:

Election of new members

Charles P. Baker, Oakland, Calif.
Parker R. Beamer, St. Louis
Warren A. Bennett, Washington
William G. Bernhard, Summit, N.J.
Herman T. Blumenthal, Louisville
Warren L. Bostick, San Francisco
William H. Carnes, Baltimore
Jacob Churg, Paterson, N.J.
Jose Curiel, Mexico City
William L. Donohue, Toronto
Carl E. Duffy, Grosse Pointe, Mich.
Thelma B. Dunn, Bethesda, Md.
Patrick J. Fitzgerald, New York
Alfred Golden, Memphis
Clinton V. Hawn, Cooperstown, N.Y.
Elwyn L. Heller, Pittsburgh
Benjamin Highman, Silver Spring, Md.
Howard C. Hopps, Oklahoma City
Pao-chang Hou, Chengtu, Szechuan, China
Lalla Iverson, Durham, N.C.
Alfred G. Karlson, Rochester, Minn.
B. H. Kean, New York
Joseph F. Kuzma, Wauwatosa, Wis.
Thomas C. Laipply, Chicago
Frederick H. Lamp, Davenport, Iowa
Raffaele Lattes, New York
Stuart Lindsay, San Francisco
Ernst Loeffler, Chicago
Alfred M. Lucas, East Lansing, Mich.
Mark E. Maun, Detroit
Frank W. McKee, Rochester, N.Y.
Joseph F. A. McManus, Birmingham, Ala.
George Milles, Chicago
John Edgar Morison, Belfast, Ireland

Richard E. Olsen, Pontiac, Mich.
Lawrence Parsons, Reno, Nev.
Machteld E. Sano, Philadelphia
Edward C. H. Schmidt, Kansas City, Mo.
Ruell A. Sloan, Arlington, Va.
Edward B. Smith, Narberth, Pa.
Cyril Solomon, New York
Paul B. Szanto, Chicago
John L. Tullis, Bethesda, Md.
Lyle A. Weed, Rochester, Minn.
O. J. Wollenman, Jr., McKinney, Texas

Reinstatement to membership of Drs. Istvan A. Gaspar, Hugh G. Grady, and Preston Kyes.

Acceptance, with regret, of the resignations of Drs. James Miller, Max Pinner, and George Shanks.

The Council announced, with deep regret, the deaths of Drs. George T. Caldwell, Mortimer Cohn, L. U. Gardner, W. P. Larson, Emanuel Libman, Ward H. MacNeal, and H. E. Robertson.

Upon nomination of the Council, the Association voted to elect the following officers:

President	MALCOLM H. SOULE
Vice-President	E. W. GOODPASTURE
Secretary	HOWARD T. KARSNER
Treasurer	ALAN R. MORITZ
Incoming Member of Council	ROBERT A. MOORE

The Council announced that it had accepted the invitation of Dr. Virgil H. Moon to hold the next annual meeting of the Association at the Jefferson Medical College, Philadelphia, and that the meetings will be held on the Friday and Saturday preceding the meetings of the Federation of American Societies for Experimental Biology.

The Council announced that the topic for the symposium in 1948 will be "Diseases of Bones" and that Dr. Henry L. Jaffe will act as referee.

For the Council, the Secretary announced the re-election of Dr. Carl V. Weller as Editor-in-Chief of *The American Journal of Pathology* for the term of seven years; the re-election of Dr. Malcolm H. Soule as Assistant Editor of *The American Journal of Pathology* for the term of one year; the election of Dr. R. Philip Custer to the Editorial Board of *The American Journal of Pathology* for the term of seven years, to succeed Dr. J. Harold Austin whose term has expired.

The Secretary read a notice from Captain G. B. Ribble, U.S.N., in reference to Naval Reserve Medical Officers.

The Secretary read a letter from Dr. O. J. Pollak inviting attendance

at the organization of The American Society for the Study of Arteriosclerosis.

The Secretary reported that the College of American Pathologists had requested appointment of two representatives of this Association to its Board of Directors. He stated that the Council had voted that participation as an association in the College of American Pathologists is considered to be outside the province of the Association. He explained that the Council had discussed this matter extensively before arriving at a conclusion. The attitude of the Council for many years has been that the Association has as its main functions the conduct of scientific meetings and of *The American Journal of Pathology*. The Council deemed it wise not to depart from this policy and took action without intimating prejudice for or against the College of American Pathologists.

SCIENTIFIC PROCEEDINGS

THE TISSUE ELEMENT IN THE ORIGIN OF NEOPLASMS. MORPHOLOGIC EVIDENCE THAT NEOPLASM ORIGIN IS PRIMARILY A TISSUE PHENOMENON. Anderson Nettleship, Detroit, Mich.

Abstract. Since there is still no accepted theory as to the mode of origin of cancer, a large number of human cancers in their earliest stages were studied in an attempt to determine if a common factor or factors were present to explain their derivation. In cases in which extremely early carcinomas were discovered, serial sections were made which included the whole neoplasm. The following types were examined: epidermoid carcinoma of the skin, epidermoid carcinoma of the cervix uteri, carcinoma *in situ* of the stomach, carcinoma *in situ* of the breast. In all cases it was possible to show that the tissue from which these neoplasms originated was in an involutionary phase and showed atrophic changes with scarring. It was also possible to demonstrate that the age of the cells in the early neoplasms, as nearly as could be determined morphologically, was the same in each neoplasm. No neoplasm was admitted to the group for study unless it showed the accepted criteria of cancer, including early invasion. Early basal cell carcinoma of the skin, melanoma, early carcinoma of the breast, and early carcinoma of the testis also were studied in order to clarify the occurrence of the asymmetrical unit formation in neoplasms. The occurrence of such units within the neoplasm, larger than the individual cell, was taken also as indicative of their multicellular origin. Cancer was shown in some instances to be limited to the area of known precancerous change, a rare probability if its origin was from a single cell.

Additional evidence was derived from sarcomas produced in tissue cultures treated with methylcholanthrene. The change from normal into cancerous cells always was found to be gradual; this is against the idea of a cell mutant being the origin of the cancer cell. On transplantation into animals, the tissue cultures produced sarcomas whose structure became increasingly anaplastic according to the length of time the original cultures had been exposed to methylcholanthrene.

The evidence strongly suggests that neoplasia comes about through the induction of cell groups at variously integrated levels: (1) widespread precancerous tissue atrophy, (2) carcinoma *in situ* with cells of identical age, (3) organization (asymmetrical) of neoplasms, and (4) induced sarcomas whose degree of anaplasticity depends upon the length of time the mother cultures were exposed to the carcinogen. Although the units are asymmetrical, abnormal cellular proliferation may occur at a number of different levels of tissue organization in tissues which are in an involutionary phase. No human cancer nor experimental cancer has ever been seen to originate from a single cell. If the above ideas are correct, research should be directed toward tissue phenomena rather than toward cellular phenomena.

THE SO-CALLED HÜRTHLE CELL TUMOR OF THE THYROID GLAND. J. B. Hazard and (by invitation) J. D. Ingle, Cleveland, Ohio.

Abstract. Eight cases of oxyphil-cell tumors of the thyroid gland were presented. Three were classified as carcinomas, five as adenomas. These tumors were of variable configuration. One patient with carcinoma died 6 months after the tumor was first observed clinically. Autopsy revealed metastases in the lung and right gluteal region. Death occurred in a second case 8 years after the tumor was first discovered and after two recurrences following operations. There were no metastases, although there was marked invasion locally. In a third instance the patient was alive and well 6 years after removal of the tumor. Of those with adenomas, two patients were alive at the end of 16 and 17 years, respectively; the others were

under observation for 1 to 3 years without evidence of recurrence. A ninth oxyphil tumor of the thyroid gland was presented with cells of parathyroid chief-cell type in the capsule and trabeculations. There was no clinical proof of parathyroid disease, but data were not available for complete evaluation. The histologic appearance of the oxyphil cells and the presence of chief cells strongly suggested parathyroid origin.

The oxyphil cells which are found occasionally in involuting thyroid tissue bear a similarity to cells of the so-called Hürthle cell tumors. Oxyphil cell neoplasms are of variable configuration and they may be benign or malignant, according to the circumstances of growth. Occasionally, oxyphil cells are intermingled with cells of undoubted thyroid origin in the same neoplasm. It is believed that the majority of the so-called Hürthle cell neoplasms originate in thyroid cells and as such should be designated as thyroid adenoma or adenocarcinoma, as the case may be, of oxyphil type. On present evidence there is an indication that these tumors, when malignant, may follow a slower course than thyroid adenocarcinoma generally and on this basis should be separately classified. In a rare instance, acidophil cell tumors of parathyroid origin may occur in the thyroid gland.

Discussion

(Dr. C. V. Weller, Ann Arbor, Mich.) It is fully in accord with the speaker's ideas that we note constantly in our Michigan material that the cells of adenomas which obviously are benign tend to acquire an oxyphilic character with the use of iodine for a long period. At the same time, the epithelium of the acini proper becomes atrophic, so that there is a reciprocal relationship. I believe fully in the thesis put forth that the Hürthle cells are not special cells, other than that they are adenoma cells influenced by the long-continued use of iodine, and may give origin to carcinomas which have similar characteristics. I wonder if anybody else has had occasion to relate this development to the use of iodine.

VERRUCOUS CARCINOMA OF THE ORAL CAVITY. Lauren V. Ackerman, Columbia, Mo.

Abstract. Verrucous carcinoma is a relatively infrequent type of neoplasm of the oral cavity, arising most frequently from the buccal mucosa and alveolar ridge of the mandible. It has a distinct tendency for papillary growth and presents considerable difficulty in diagnosis on biopsy. It spreads slowly over a wide area and tends to invade contiguous soft tissues, growing through the skin of the cheek, and into the mandible. In spite of extensive local invasion, regional lymph nodes are almost invariably spared. Its gross and microscopic characteristics are typical.

Discussion

(Dr. Harold L. Stewart, Bethesda, Md.) I should like to ask concerning possible etiologic factors in these cases. Khanolkar of Bombay, India, has reported a lesion somewhat resembling the lesion described, which he called "Chutta cancer." The natives smoke a local type of cigar, the "Chutta," and put the burning end in their mouths. They get cancer of the hard palate. I am curious to learn whether any similar etiologic factor was known in these cases of Dr. Ackerman.

(Dr. Ackerman) I am glad Dr. Stewart asked that question. We have been impressed by the fact that Missouri farmers frequently chew tobacco. In my series, 18 of the group of 31 chewed tobacco, and, as you recall, the majority of the lesions were in the region of the buccal mucosa where the chewing tobacco is usually kept. It is also interesting that with an average age of 67, there were 5 females, of whom one was 41, and this one, like some other Missouri farmers' wives, also chewed tobacco. Chewing of tobacco, therefore, may be of some etiologic significance. About one-third of these patients also had leukoplakia and a great many of them had very poor dental hygiene.

About a year ago I talked with a pathologist from the Tata Institute, Bombay, India. After looking at these lesions, he thought that they were not similar to any he had seen in India.

CANCER CELLS IN BRONCHIAL SECRETIONS. Peter A. Herbut, Philadelphia, Pa.

Abstract. In order to establish an earlier diagnosis in carcinoma of the lung, we have developed a method of examining bronchial secretions for neoplastic cells. In the course of an ordinary bronchoscopic examination, secretions are secured from the bronchus that drains the area containing the suspected cancer. If there are no secretions present, the area is washed with physiologic saline solution and the washings are aspirated. The material obtained is sent to the laboratory where thin smears are prepared by the Papanicolaou technic and examined for cancer cells. In most instances these can be precisely identified. In general, the diagnostic criteria consist of: (1) Changes in shape and size of cells. Normal epithelial cells are relatively small columnar, cuboidal, or occasionally round. The first two bear cilia. Cancer cells are not ciliated. They vary in size from normal to ten times the normal diameter, and they are of every conceivable shape. (2) Cytoplasmic alterations. Normally, the cytoplasm stains light green and is moderate in amount. Cancer cells show scanty to abundant pink, orange, gray, dense or granular cytoplasm. (3) Changes in the nuclei. Normally, these are round, oval, vesicular, and relatively small. Neoplastic cells have an increased nuclear-cytoplasmic ratio. The nuclei are round, oval, or extremely bizarre and irregular. They are either so intensely hyperchromatic that no internal structure can be discerned or they are very lightly stained and washed out. The latter are always large and have distinct borders, clumped nucleoplasm, and often prominent nucleoli.

To date we have examined 525 preparations. In this group there were 118 cases of cancer of the lung. A cytologic diagnosis of carcinoma by the method described was rendered in 105, or 89 per cent. In this same group of 118 cases it was possible to remove tumor bronchoscopically for histologic study in only 52 cases, or 44 per cent. In an additional 23 cases there was bronchial stenosis or distortion, but a tumor was not visualized. In 32 cases, or 27 per cent of the total in which cancer cells were found in the secretions, bronchoscopic examination was negative. A false positive diagnosis was rendered in only 4 cases.

As a result of this work our preoperative morphologic diagnoses of carcinoma have been doubled and our patients are being operated upon much sooner than was heretofore possible.

Discussion

(Dr. Jacob Werne, Jamaica, N.Y.) In those cases in which there was a discrepancy between the finding on biopsy and that by smear, what other evidence is there, either clinical or post-mortem, to substantiate the correctness of the diagnosis made after examination of the smear?

(Dr. William Boyd, Toronto, Ont.) A week ago I was in Montreal and I saw a demonstration by Dr. Mathews of the Montreal General Hospital in which the technic was a little different, but the results were certainly as striking. Mathews used sputum rather than bronchial secretions. He had previously used bronchoscopic secretion, but was more satisfied with sputum examination. His technic was to collect a 24-hour specimen of sputum, put it in a muslin bag, immerse it in Bouin's fixative, which caused it to contract greatly, and cut sections of the material. He stained them with hematoxylin and eosin, and the color photographs were quite dazzling in their beauty. I have never seen anything more striking. One glance enabled one to tell that these preparations were malignant. They were cases which had been proved by operation or autopsy to be carcinoma, and the sputum diagnoses were 64 per cent correct.

My last point is, what is the advantage of the Papanicolaou method? Dr. Mathews obtained equally good results with hematoxylin and eosin.

(Dr. B. Earl Clarke, Providence, R. I.) I believe there were 500 cases studied, and 118 were proved positive. Will Dr. Herbut please tell us the results of the other 382 cases? How many of these were positive by smear?

(Dr. William H. Harris, New Orleans, La.) Would Dr. Herbut kindly define and describe an "unmistakable cancer cell"?

(Unidentified discussant) May I inquire as to whether there were any errors in the diagnosis when a diagnosis of cancer was made, and how often these occurred?

(Dr. Herbut) I am not quite sure that I understood the first question. Every case in which a positive tissue specimen was secured bronchoscopically was found to be positive by the smear method. Is that what you mean?

(Dr. Werne) There was a discrepancy between the finding on biopsy and that by smear in some cases; what was the other clinical or post-mortem evidence to substantiate the correctness of the smear diagnosis?

(Dr. Herbut) In each one of these cases the diagnosis was made at thoracotomy and proved histologically. Every one of these 118 cases was a proved case, either by autopsy, by thoracotomy, or pneumonectomy; histologically, at any rate.

As far as sputum is concerned, I have not tried the method which Dr. Boyd outlined. In about 15 cases in which we were able to make a diagnosis on secretions obtained bronchoscopically, I did, however, examine the sputum faithfully, sometimes as many as a dozen different specimens, and in not one was I able to find a single cancer cell.

(Dr. Boyd) Did you examine them by section?

(Dr. Herbut) No.

As far as the Papanicolaou method is concerned, I think it has a distinct advantage. Squamous cell carcinoma is the most common type of cancer of the lung, and by this method one finds neoplastic cells in secretions staining pink, orange, or red. One glance at a smear will tell you it is carcinoma.

I said there were 525 preparations made. Naturally, many of the cases suspected of being carcinomatous turned out to be bronchiectasis, abscesses, tuberculosis, etc. These constituted the other 407 cases.

As to "unmistakable neoplastic cells," if you had studied these smears as we have, and in each one of these cases made smear preparations of the tumor, and compared the smears of the tumor with the smears obtained bronchoscopically, you would see that the cells are unmistakably neoplastic cells. One can superimpose one cell upon the other.

The errors in diagnosis: As you saw, our diagnosis was correct in 89 per cent of the cases. In 11 per cent we were unable to make a diagnosis by the smear method. That percentage has decreased, for in the last 60 cases we have missed only 3.

We have made only 4 over-diagnoses, and these were in the earliest part of the study. Three were cases of pulmonary abscess in which there was metaplasia of the epithelium. In each case there was only one nest of rather regular squamous cells. With added experience these offer no difficulty, for since then we have picked up several similar cases which we did not call neoplastic. In one case I made a diagnosis of carcinoma by finding a single cell, and when I look back at that now I do not think it was justified.

PRECANCEROUS LESIONS OF FORESTOMACH OF MICE INDUCED BY 20-METHYLCHOLANTHRENE AND 1,2,5,6-DIBENZANTHRACENE. Harold L. Stewart and (by invitation) Egon Lorenz, Bethesda, Md.

Abstract. Mice were given, instead of drinking water, an aqueous mineral oil emulsion containing 1,2,5,6-dibenzanthracene or 20-methylcholanthrene. More than

50 per cent of the animals developed squamous cell carcinoma of forestomach and precancerous lesions. The majority of the animals showed, singly or in combination, acanthosis of the squamous epithelium, hyperkeratosis, umbilicate foci with dyskeratosis and solitary or multiple papillomas. The areas of acanthosis and hyperkeratosis appeared as white, firm, corrugated projections from the mucosa, which were widespread, largely occluded the lumen of the viscus, and frequently showed dyskeratosis. At the keratinized margin overlying an area of dyskeratosis, there was usually a focal inflammatory area with a linear break in the keratin above. In this area there was loss of the granular cells and parakeratosis; some of the anuclear parakeratotic cell-like bodies were distended with fine acidophilic refractile granules, or were completely filled by deeply stained or pale acidophilic hyalin. These changes were observed in the umbilicate lesions and in the papillomas.

Carcinoma *in situ* was characterized by the presence of one, several, or all of the precancerous lesions described, spread over a wide segment of the forestomach and exhibiting multicentric development of carcinoma.

These specimens of forestomach showed diffuse and focal inflammatory cell infiltration of the lamina propria, the submucosa, and the subserous and intermuscular connective tissue, and, less frequently, the muscle coats. Amyloid was almost always present in the lamina propria of the forestomach, occurring in the walls of the vessels and being most marked in the tips of the papillary processes and in the cores of the papillomas. In contrast, the submucosa was almost always free of amyloid. Degenerative changes in the collagen and reticulum of the lamina propria and submucosa were regularly observed in association with the inception of dyskeratosis of the overlying epithelium.

Discussion

(Dr. Anderson Nettleship, Detroit, Mich.) I would like to ask in regard to the diet of these animals. It is well known that a vitamin-A deficiency produces the same type of lesion. Did these animals eat well? The second thing I would like to speak about is the widespread character and breakdown of these precancerous lesions. I would like to ask Dr. Stewart if he can tell about the time of occurrence of the atrophy and the precancerous lesion in their relationship to each other—which came first, or were they simultaneous, and how long did it take for the neoplastic changes to develop?

(Dr. Ruth Silberberg, St. Louis, Mo.) I would like to ask if there were any strain differences in the susceptibility to this type of tumor, and also whether there is any parallelism in the susceptibility to this type of tumor and to skin tumors produced by carcinogenic agents?

(Dr. Emmerich von Haam, Columbus, Ohio) Were there any metastases?

(Dr. Alfred Angrist, Jamaica, N.Y.) I wonder whether Dr. Stewart will tell us about the diet these animals were kept on. In a series of experiment some years ago, and there was an earlier description of this type of material on vitamin-A and B deficiency diets, this lesion occurred spontaneously in the forestomach of rats. It would be interesting to know whether the diet would have any effect as to the sequence in time of the lesion and the degree of its appearance and development.

(Dr. Stewart) We kept our mice on dog chow. These animals were toxic; they did not gain weight as well as the controls; however, it was possible to keep them living for as long as 13 months. They will develop anasarca if they are on the emulsion for a long time, and, interestingly enough, the anasarca disappears if they are taken off the emulsion regime and put on water. The diet is very important. I would not be surprised to find that they did have a vitamin A deficiency because of the mineral oil which they ingest. We have studied a number of animals on vitamin-A deficiency diets. A number of years ago, Andervont kept various strains of mice on vitamin-A deficient diets and, although we saw papillomas, we never saw a carcinoma of the forestomach, or precancerous lesions. In our con-

trol mice we saw hyperkeratosis and acanthosis, but in these there was no evidence of dyskeratosis, and no carcinoma developed. The question of the relation of the diet to this sort of thing is very interesting and requires experimentation.

The earliest tumor appeared at autopsy at 87 days, the earliest precancerous lesion at 78 days. Our experiment was not designed to determine just exactly when they appeared. It was designed to induce carcinoma and we tried to keep the mice living as long as possible to see whether metastases would occur. As a result of our work with 90 mice in this experiment, we are not prepared to say exactly when these lesions first develop. Of these 90 mice, 57 developed squamous cell carcinoma of the forestomach, and about the same number showed precancerous lesions. Some of the mice with carcinoma showed precancerous lesions in other parts of the forestomach. Forty-six per cent of the mice with carcinoma of the forestomach showed metastases, or local extension, to pancreas, spleen, peritoneum, diaphragm, regional lymph nodes, liver, kidney, genital omentum, and lungs.

There is a strain susceptibility. In the ABC backcross mice, we never obtained squamous cell carcinoma of the forestomach with olive oil emulsions of 1,2,5,6,-dibenzanthracene, and yet in strain A mice we obtained this lesion.

We have not investigated the possibility of any parallelism between induction of carcinoma of the forestomach and induced skin tumors.

The relationship between the precancerous changes and the atrophy of the mucosa of the forestomach requires further study.

THE DISTRIBUTION OF PARIETAL CELLS IN GASTRIC DISEASE. William A. Meissner, Boston, Mass.

Abstract. There is now general agreement that the hydrochloric acid of the stomach is secreted, directly or indirectly, by the parietal cells of the gastric mucosa. Although the determination of gastric acidity has become almost a routine procedure in the study of stomach disorders, particularly peptic ulcer and cancer, the state of the cells which secrete the acid has been left largely to conjecture.

An attempt was made to determine whether there are quantitative or qualitative differences in the parietal cells in conditions in which there is usually hyperacidity (peptic ulcer) as contrasted with conditions in which the acid is low or absent (gastric cancer). A series of 200 surgically resected stomachs, removed because of gastric cancer or peptic ulcer of the stomach or duodenum, was examined. Multiple sections were taken from each stomach, insofar as possible from the same representative areas, and the histologic appearance of the parietal cells was noted; likewise, an estimate was made as to whether the cells were abundant or moderate to few in number in each section. The parietal cells in all specimens diminished in number as the pylorus was approached and were fewer along the entire lesser curvature when contrasted with the opposite areas on the walls or greater curvature. The only quantitative change of significance was that many cases of carcinoma showed a marked diminution in the total number of parietal cells in the body and fundus, whereas they were diminished less frequently in these areas in ulcer. This diminution, however, was not a constant finding in cancer, and many cases with cancer and complete anacidity showed abundant parietal cells; no stomach showed a complete absence of such cells. As to qualitative changes in the individual cells, as seen in routine stains, there were no specific nuclear or cytoplasmic alterations which could be correlated with hyperacidity or anacidity.

The cause of diminished secretion of gastric acid cannot be explained alone on quantitative or qualitative morphologic changes in the parietal cells. Further work is warranted to determine such cause or causes since the problem is of more than academic interest. It has been frequently noted that anacidity may precede the onset of carcinoma of the stomach.

Discussion

(Dr. Howard C. Hopps, Oklahoma City, Okla.) I would like to know whether Dr. Meissner has observed gastric cancer in which the origin appears to be in the parietal cells, and, if so, what was the character of this neoplasm as far as acid secretion was concerned.

(Dr. Meissner) I have never observed in our material a carcinoma which could be definitely proved to arise from the parietal cells of the stomach. There are many gastric carcinomas with cells similar to parietal cells, but I believe in most instances they are merely degenerated tumor cells. Carcinoma cells often become acidophilic when they degenerate.

PRIMARY TUMORS OF THE PERITONEUM. Louisa E. Keasbey (by invitation), Los Angeles, Calif.

Abstract. No comprehensive review of tumors arising from the lining cells of the peritoneal cavity is to be found in the Anglo-American literature. These tumors are rare. Not only is misapprehension prevalent as to their structure, types, and clinical course, but there is incredulity as to their existence.

The purpose of this paper is to present a classification of the primary tumors of the peritoneum with a brief review of the literature and a report of 8 representative cases.

I. Primary carcinoma of the peritoneum (4 cases, one of the tunica vaginalis testis).

A. The papillary ascitic type (2 cases): This tumor begins as small nodules (which were seen and excised at laparotomy), sparsely scattered over the peritoneal surface. This is followed by a period of extreme recurring ascites. Later the peritoneal cavity becomes obliterated and ascites disappears. The tumor tends to spread over the entire surface of the peritoneum and at necropsy the peritoneal cavity is often found totally obliterated with such dense adhesions that no cleavage lines can be found. The tumor replaces no organ, does not metastasize, and is distinguished by limiting itself to uniform superficial invasion. Its histologic picture is considered distinctive.

B. The pseudomucinous type (1 case): Tumors of this type are said to present a more glandular structure than those of type A, but in the case reported this was not striking. The course of the disease and the microscopic and gross pictures are similar to those seen in type A, but the abundant peritoneal fluid is thick and mucinous or pseudomucinous.

C. The multicystic ascitic type: a representative case is discussed, but it is felt that these tumors are probably of retroperitoneal lymphangiomatous nature and are neither carcinomas nor of primary peritoneal origin.

II. Benign tumors of the peritoneum.

A. Primary peritoneal papillomatosis (2 cases, one involving the abdomen, but studied only at laparotomy; one involving the abdominal peritoneum, pleura, and tunica vaginalis testis).

B. Mesothelial adenoma (2 cases): Small benign serosal tumors, described independently in this country and in Italy as involving the serosal surfaces of the genitalia.

III. Primary tumors of the tunica vaginalis testis—an extension of the peritoneum (3 cases: carcinoma, papilloma, and adenoma).

THE BENIGN GIANT CELL TUMOR OF TENDON SHEATHS. AN EXAMPLE OF SCLEROSING HEMANGIOMA.* Lee N. Foster (by invitation), Boston, Mass.

Abstract. The present study reviews 41 tumors emphasizing sequential tissue changes. Most striking of these is a progressive increase in fibroblastic stroma.

* *Am. J. Path.*, 1947, 23, 567–583.

Specimens in which little fibrosis is evident are highly vascular, the blood vessels being separated from one another by a cellular tissue. As stromal overgrowth destroys this vascular pattern, intravascular cells phagocytose lipid and hemosiderin. Despite regressive features induced by sclerosis, mitotic figures appear in the intervascular cells, defining the lesion as a true tumor. The activities of the intervascular cells as well as the manner in which the sclerosing process is centered about blood vessels suggest that the lesion is a sclerosing hemangioma.

Discussion

(Dr. D. Murray Angevine, Madison, Wis.) I should like to point out that the sub-synovial tissue of the tendon sheath is a very vascular tissue. I would like to ask Dr. Foster if he has ever seen an angioma of the tendon sheath. They frequently occur around joints. I would like to be sure that angiomata occurred before we classify these lesions as sclerosing angioma. We do have angiomata in the skin and that is the place where sclerosing angiomata occur.

(Dr. Alfred S. Giordano, South Bend, Ind.) I should like to ask whether Dr. Foster has ever seen a giant cell tumor such as that demonstrated in a sclerosing hemangioma of the skin.

(Dr. Louis Lichtenstein, New York, N.Y.) We have been aware for some time of the thesis entertained by Dr. Foster and his colleagues, and I followed his presentation with a great deal of interest. Frankly, I am no more convinced of its soundness now than I was before. This lesion is not unique in the tendon sheath, and has its anatomic counterpart in the synovial lining and sub-lining connective tissue of joints, and also of bursae. Some years ago Drs. Jaffe, Sutro, and I described these lesions with the idea that we were dealing with some peculiar inflammatory condition which we designated pigmented villonodular synovitis and bursitis, referring to its tendon sheath expression as pigmented nodular tenosynovitis. Thus, the nodules springing from the modified synovium in cases of pigmented villonodular synovitis may be indistinguishable histologically from the tendon sheath nodules in question. The early stages of evolution of the condition can be observed to better advantage in the synovial and bursal lesions than in the tendon sheath nodules, since the latter are generally far along in their development when they are extirpated, and already show more or less extensive fibrosis. If one examines the early stages histologically, one observes, to be sure, that the lesion is rather vascular, but its most striking feature is intense proliferation of large polyhedral cells, which may be so compact as to suggest a neoplasm. These cells, however, are macrophages by function, and they take up hemosiderin pigment granules and, in some cases, lipids as well. Eventually they tend to be replaced by collagenized fibrous connective tissue. Now, some of these macrophages appear to be derived from the adventitial cells of blood vessels, but if that establishes the nature of the lesion as a hemangioma, then, to be consistent, one would have to call practically any inflammatory lesion a hemangioma.

(Dr. Helen Ingleby, Philadelphia, Pa.) Have any of the tumors been stained for reticulin? If characteristic reticulin formation was present in the earlier tumors, it might help clarify the etiology.

(Dr. Foster) In answer to Dr. Angevine's question regarding the relationship of such lesions to synovial membranes, this question has been partially answered by Dr. Lichtenstein. Such lesions do occur in relationship to the synovial membrane.

In answer to Dr. Giordano's question, I have seen such giant cell tumors in the skin.

In answer to Dr. Lichtenstein's discussion, I believe our observations are quite similar, but the discrepancy arises in our opinion as to whether these are progressive growths. In studying these tumors I have felt I could find evidence of continuous growth throughout the sequences of sclerosis, which led me to believe

that the lesion was a true tumor. He apparently has not observed this, or has not felt it important in interpreting the nature of the lesion, and so for this reason I believe we diverge in our final conclusions.

A New Form of Granulomatous Disease in Man Characterized by Intracellular Parasitism Due to an as Yet Unidentified Acid-Fast Organism.

J. T. Cuttino, A. McCabe, and M. L. Weil, Jr. (by invitation), Durham, N.C.

Abstract. The problem considered in this report was presented by a 34-months-old white girl whose illness began 4½ months prior to death. A mass in the abdomen was diagnosed lymphosarcoma. Biopsy of an enlarged node showed many large macrophages containing great masses of acid-fast organisms. The infant died 2½ months later after a course resembling inanition. At autopsy there was massive enlargement of the lymph nodes of the mesenteric and retroperitoneal groups with moderate enlargement of the left subclavian and mediastinal nodes, enlarged spleen, and ulcers of the colon. Microscopically, this enlargement was due to great proliferation of macrophages which contained many acid-fast organisms. There were additional foci of these parasitized macrophages in the liver, lungs, and pancreas. The organism was obtained in pure culture from the lymph nodes and spleen. It is pathogenic for, but not lethal to, guinea-pigs, mice, and rats. It is nonpathogenic for rabbits, frogs, chickens, and goldfish. Infection is uniformly successful by subcutaneous, intraperitoneal, and intravenous injection; by ingestion; and by instillation in the eye. The lesions produced consist of a central zone of necrosis surrounded by a broad zone of epithelioid cells. In about 10 per cent of instances infection becomes systemic, but intracellular parasitism is not as marked in experimental animals as in the human being. Organisms can be reclaimed in pure culture from these lesions. There is spontaneous healing, and a tuberculin type of sensitivity is produced. Morphologically, the organism is a gram-positive, strongly acid-fast, short filamentous, branching organism. Culturally, it produces a yellowish, moist colony in 3 to 5 days on Petragnini, Bordet-Gengou, and other media including Sabouraud's, at room temperature. On van Tieghum cell mounts, mycelia and branching appeared in 2 to 5 days. There are no aerial mycelia. This organism appears to be an actinomycete, specifically a Nocardia. However, no description of an identical organism has thus far been encountered. The histologic reaction is unusual in that it is entirely macrophagic. The intracellular parasitism is most closely allied to that of leprosy and Johne's disease of cattle, but in these diseases there are other reacting elements.

Discussion

(Dr. Roger D. Baker, Birmingham, Ala.) I should like to ask how this case differs from reported cases of nocardiosis or streptothricosis.

(Dr. Anderson Nettleship, Detroit, Mich.) I wonder if Dr. Cuttino could find any difference between this and Johne's disease? You can hardly see these macrophages with the punched-out central hole without thinking of Johne's disease of cattle. I wonder if he tried to prove it to be Johne's disease, since that would be the logical thing. I suppose the child lived in the country. Was there any evidence of Johne's disease in the cattle in the region from which she came?

(Dr. Cuttino) The lesion of nocardiosis is of a more purulent type, and in some species there is granule formation. In our human case, necrosis was not a feature. In experimental animals the lesion is somewhat similar, but shows extensive necrosis.

In reply to Dr. Nettleship, we have had opportunities to compare this with Johne's disease by means of sections we obtained from the Bureau of Animal Industry. In those cases the lesion was confined to the gastrointestinal tract, and there was not this massive display of organisms. The organisms were smaller,

and we have used a culture of the organism of Johne's disease, also obtained from the Bureau of Animal Industry, and were unable to produce the lesion in experimental animals.

MODIFICATIONS OF TUBERCULOUS LESIONS IN PATIENTS TREATED WITH STREPTOMYCIN. Curtis M. Flory and J. W. Correll (by invitation), and J. G. Kidd, New York, N.Y.

Abstract. Five patients with miliary tuberculosis who had been intensively treated with streptomycin were examined post-mortem. In all cases there was evidence that the tuberculous lesions had been modified by the therapy.

In one patient, for example, extensive miliary tuberculosis of the lungs, demonstrated roentgenologically, had resolved completely after about 35 days of therapy; an associated tuberculous meningitis at first had responded to the drug given intrathecally but eventually had relapsed, causing an internal hydrocephalus and death 297 days after the initial diagnosis had been made. Gross examination of the lungs post-mortem revealed no trace of the miliary lesions, and cultures of the lungs for acid-fast bacteria were negative, though a healed primary complex was found. Microscopically, there were numerous small scars consisting of loose fibrous tissue scattered irregularly throughout all lobes of the lungs; these revealed no evidence of active tuberculosis, and they were interpreted as being the healed remains of miliary nodules. Similar scars were found post-mortem in the lungs of 3 additional patients who had responded temporarily to the therapy, though in these cases there was widespread recurrence of active tuberculosis along with focal fibrosis throughout the lungs.

In still another patient, whose miliary tubercles had disappeared following the initiation of drug therapy, as indicated by x-ray examinations, but had reappeared later when the infecting bacterium had become drug-fast, very many large miliary tubercles were visible in the lungs on post-mortem examination. Microscopically, these lesions were discrete and spherical, and each was composed of a thick fibrous capsule arranged concentrically about an area of granulomatous inflammation and caseation that often had broken through the fibrous barrier and more or less surrounded the latter. These lesions contained great numbers of acid-fast rods, as special stains revealed, and their "inside-out" character suggested that the bacteria responsible for them had been held in check for a time and then gained overwhelming impetus, perhaps when they had become resistant to the drug.

Post-mortem studies of the central nervous system in these 5 patients are still under way in the laboratory of Dr. Lewis D. Stevenson. The findings thus far, however, make it plain that the tuberculous lesions in at least 3 of the patients had been modified by streptomycin therapy.

STREPTOMYCIN-RESISTANT TUBERCLE BACILLI: EFFECT OF RESISTANCE ON THERAPEUTIC RESULTS. William H. Feldman, A. G. Karlson (by invitation), and H. Corwin Hinshaw, Rochester, Minn.

Abstract. Objective: To determine if infections produced by tubercle bacilli having a marked *in vitro* resistance to streptomycin would respond to streptomycin therapy.

Procedure: Tubercle bacilli with normal sensitivity to streptomycin were obtained from a patient before treatment with streptomycin was started. A culture resistant to streptomycin *in vitro* was obtained from the same patient after treatment for 4 months with streptomycin. Two experiments were done concurrently. In one, guinea-pigs were infected with the sensitive culture and in the other experiment a similar group of guinea-pigs was infected with the resistant culture. Twenty days after inoculation 10 animals in each experiment were started on treatment with streptomycin. Treatment was continued daily until all of the untreated controls had died (approximately 23 weeks).

Results: The disease in the animals infected with the streptomycin-sensitive culture responded favorably to therapy. However, in 3 of 10 animals active lesions of recent origin were present. Streptomycin-resistant tubercle bacilli were obtained from each of these 3 animals. The disease in the animals infected with the streptomycin-resistant culture failed to yield to therapy. In this instance the amount and character of the tuberculosis in the untreated controls and in the treated groups were comparable.

Conclusions: Infections in guinea-pigs induced by tubercle bacilli resistant *in vitro* to streptomycin are refractory to therapy with this antibiotic.

Discussion of Papers by Drs. Flory, Correll, and Kidd; and by Feldman, Karlson, and Hinshaw

(Dr. Richard H. Follis, Jr., Baltimore, Md.) I should like to ask what happened to the visceral lesions, that is, the lesions in the spleen, liver, and bone marrow, of the patients studied with miliary tuberculosis.

(Dr. M. H. Soule, Ann Arbor, Mich.) I would like to ask if there are any colonial or tinctorial differences, or differences in virulence that go hand in hand with resistance or sensitiveness to streptomycin.

(Dr. J. W. Correll) As far as visceral lesions in these patients were concerned, in a few cases we did find small hyaline scars in the liver, such as have been described by Dr. Baggenstoss. In one patient we found very fibrotic lesions in the kidneys. However, in other cases we found no definite evidence of modification.

(Dr. Feldman) In our experience, Dr. Soule, we have been unable to detect any significant colonial or tinctorial differences in the strains that were resistant or highly susceptible to streptomycin. From the standpoint of virulence it is my impression that possibly a diminution in virulence is associated with a state of resistance. However, we have little factual evidence on this question.

DEGENERATION AND NECROSIS OF NEURONS IN EIGHTH CRANIAL NUCLEI CAUSED BY STREPTOMYCIN. Lewis D. Stevenson, E. C. Alvord, Jr., and J. W. Correll (by invitation), New York, N.Y.

Abstract. Liquefaction necrosis of neurons, sometimes with dropping out of cells, was found post-mortem in the ventral cochlear nuclei in 5 patients who became partially or completely deaf while receiving large amounts of streptomycin for the treatment of tuberculosis. Similar changes were present in the inferior vestibular nuclei in 1 of 2 patients in whom these structures were studied. Other cranial nerve nuclei were normal in all cases. While all of the patients died with tuberculosis and all manifested varying degrees of tuberculous involvement of the cranial nervous system, there was no clinical evidence that the function of cranial nerves other than the 8th had been disturbed in any case. In one there was softening of a part of the basis of the pons immediately below the ventral cochlear nucleus, this change being unilateral, whereas the neuronal degeneration presumably due to streptomycin was bilateral.

Three dogs were given large doses of streptomycin and developed marked weakness and incoordination, although no deafness was noted. One died on the 9th day with necrotizing renal arteriolitis and glomerulitis, and the other two were sacrificed on the 28th day. All showed lesions in the ventral cochlear nuclei bilaterally, with liquefaction and neurons similar to that found in the clinical cases, but no apparent dropping out of cells. In the dog that died of renal disease, peculiar clumps of Nissl-like material were present in the cytoplasm of the cells of the ventral cochlear nuclei.

Apparently, streptomycin can cause in man and experimental animals a specific destructive effect on the neurons of the 8th cranial nuclei, especially the ventral cochlear nuclei, and possibly the inferior vestibular nuclei. These changes would

seem to be sufficient in some of the clinical cases to account for deafness and perhaps for vestibular dysfunction also.

Discussion

(Dr. Virgil H. Cornell, Washington, D.C.) I would like to ask whether any of these five patients demonstrated allergy to the drug during their early treatment and had to be desensitized. I ask this because of information transmitted to me by Dr. Romansky, and I wish to give him full credit for it. I understand that he has found the percentage of patients with 8th nerve injury to be very high following the use of streptomycin in tuberculosis. However, none of five patients, who had been allergic to the drug and whom he therefore had to desensitize before treatment was begun, showed any of these symptoms. I think it is a most important observation; it is one, I am sure, that he is going to follow up in the future, but I felt it was important enough to bring before this society for consideration.

(Dr. Cecil A. Krakower, Chicago, Ill.) I should like to ask whether the authors examined the cochlear apparatus or the semicircular canals in this connection, since we know that certain drugs can affect the end-organs in the internal ear.

(Dr. Archie Baggenstoss, Rochester, Minn.) I should like to ask if determinations were made of the streptomycin content of the brain. We reported two cases last year in which the cerebrospinal fluid became negative, and at autopsy no cerebrospinal meningitis was found, yet extensive tuberculous lesions were found in the brain. In one case the brain was analyzed for streptomycin content, and absolutely none was found.

(Dr. Alvord) In regard to the question of allergy, only one patient showed any signs of allergy, a fairly marked eosinophilia. Neither he nor any of the others was desensitized.

Concerning the question of the examination of the cochlear apparatus, we have not examined that; but as to the possibility of this being a retrograde damage, I would like to point out that the cells in the ventral cochlear nucleus are secondary neurons, and not those that supply the fibers in the 8th nerve or those to the internal ear.

As to streptomycin concentration, we have not analyzed that factor either.

THE INFLUENCE OF 3,3′-METHYLENE-BIS-(4-HYDROXYCOUMARIN) UPON STREPTOCOCCUS INFECTION IN RABBITS. George R. Thuerer (by invitation) and D. Murray Angevine, Madison, Wis.

Abstract. Prothrombin levels were determined on rabbits prior to and following the oral administration of dicoumarol. There was considerable variation in the response of the animals to dicoumarol. Some were entirely resistant, whereas the prothrombin time of those that responded was 3 or more minutes in contrast to the normal time of 4 to 7 seconds. These were paired with normal rabbits and both groups were infected with a single intracutaneous injection of virulent *Streptococcus haemolyticus*. The local lesions were measured daily, prothrombin levels were determined during the course of the infection, and blood cultures made when an animal died or was killed.

The skin lesions on the dicoumarolized animals were, with few exceptions, larger, more diffuse and more spreading than those of the controls. Some of the animals were killed at intervals after infection whereas others were followed until the animal died or the infection subsided. Of 14 rabbits that responded to dicoumarol, 7 developed a positive blood culture, 5 of them dying of septicemia. Only one of 15 control animals developed septicemia.

Histologic examinations were made of the skin lesions and draining lymph nodes of 10 treated and 10 control rabbits to determine and compare the amount of fibrin in the two groups. There was definitely more fibrin in sections from the control

group. Dicoumarol-treated animals that failed to respond by prolonged prothrombin time showed fibrin in similar amounts to the controls.

These experiments strongly indicate that the lack of fibrin formation due to an interference with the mechanism of coagulation in dicoumarolized animals may play a significant rôle in the more extensive spread and greater invasiveness of the infection in treated, as in contrast to control, animals.

Discussion

(Dr. Richard H. Follis, Jr., Baltimore, Md.) I should like to ask if this was a fibrinolytic strain, and suggest that more clear-cut observations might be made if a nonfibrinolytic organism was used.

(Dr. Angevine) This is a fibrinolytic strain, and is very virulent. We plan to use other organisms, but I believe it is more important to use other species of organisms first than to use other strains of streptococcus.

THE NORMAL HUMAN ADRENAL CORTEX AND ITS RESPONSE TO ACUTE DISEASE. Norman Zamcheck (by invitation), Boston, Mass.

Abstract. "Post-mortem autolysis" does not account for most of the changes found in the human adrenal cortex at autopsy. A systematic review of large numbers of cases has shown that histologically "normal" adrenal cortices are found when normal persons are killed instantly by violence. Characteristic and readily recognizable histologic changes are found in the adrenal cortices of patients dying of a variety of acute conditions, including infections, traumatic injury, poisoning, burns, and others. The pathogenesis of these changes as found in a group of men dying of diphtheria is presented.

THE PATHOLOGY OF ADDISON'S DISEASE: ADRENOCORTICAL CONTRACTION. Nathan B. Friedman, Washington, D.C.

Abstract. Between December, 1941, and December, 1946, pathologic material from 15 patients who exhibited adrenocortical contraction at autopsy was received at the Army Institute of Pathology. During the same period specimens from only 10 patients with Addison's disease caused by tuberculosis were accessioned. Prior to World War II, only 6 cases of adrenocortical contraction had been studied at the Institute, as contrasted with 21 cases of tuberculous Addison's disease.

The clinical syndrome was in many instances so atypical and confusing that the diagnosis of adrenal insufficiency was not entertained, particularly when the disease ran a short, fulminating course. Acute gastro-enteritis, poisoning, ruptured peptic ulcer, coronary occlusion, psychosis, intracranial hemorrhage, and myasthenia gravis were all simulated. The danger of temporizing with the medical emergency of adrenal insufficiency was underlined by the repeated occurrence of sudden collapse and death.

The morphologic picture in the 21 cases of full-blown adrenocortical contraction ranged from that of pure destructive atrophy, or collapse, of the cortex to that in which the regeneration of cortical cells and nodules in atypical patterns overshadowed and masked the underlying atrophy. Although the early stage of "atrophy" is rarely encountered in material obtained at autopsy, the Institute files contain 4 examples of cortical degeneration, necrosis, and inflammation so pronounced that one could conceive of their being the precursors of adrenocortical contraction.

The lesions in adrenocortical contraction differ strikingly from those caused by vascular occlusion, syphilis, and tuberculosis. They bear a strong resemblance to the lesions of necrotizing hepatic injury and its sequelae.

Discussion of Papers by Drs. Zamcheck and Friedman

(Dr. Sol Roy Rosenthal, Chicago, Ill.) In a study of the adrenal gland in chronic diseases such as pulmonary tuberculosis, I noted the changes described by

Dr. Zamcheck plus a more advanced stage of hyperplasia to form nodes in the cortex, especially the capsule. In 71 cases drawn from the Bruns General Hospital and the Army Institute of Pathology, it was found that there were 1.18 nodules per case in comparison to 0.38 nodule per case in those dying from traumatic sudden death. The average length of the disease in the cases studied was 9.5 months.

(Dr. William Boyd, Toronto, Ont.) I noticed that in one of the slides which Dr. Friedman showed he had two cases of unilateral contraction. These were from cases of Addison's disease. I wonder what the other adrenal was like.

(Dr. Howard T. Karsner, Cleveland, Ohio) How does the Army Institute of Pathology make a diagnosis of syphilis of the adrenal?

(Dr. Averill A. Liebow, New Haven, Conn.) Has Dr. Friedman noticed any inclusion bodies in the adrenals?

(Dr. Russell L. Holman, New Orleans, La.) I would like to ask Dr. Friedman whether any of the patients had a history of an acute episode which might have been associated with necrosis of the adrenal. There are reports of clinical cases of Waterhouse-Friderichsen's syndrome which recovered following the use of adrenal cortical hormone. Do you have such a history in any of the cases of "contraction" of the adrenal?

(Dr. Howard C. Hopps, Oklahoma City, Okla.) I should like to ask Dr. Zamcheck whether he believes that these changes in the adrenal cortex in relation to so many conditions play a direct rôle in the peripheral vascular collapse that so often characterizes the disease.

(Dr. N. Goormaghtigh, Ghent, Belgium) It is a great pleasure for me to hear Dr. Zamcheck's work. I made similar observations about 20 years ago, by studying material in the first World War. The reason why I have not continued to be interested in this problem is that so many factors influence the structure and the behavior of the adrenal cortex, and I think that it is a work for the future to dissociate the different factors which act on the suprarenal cortex.

(Dr. Zamcheck) Dr. Goormaghtigh has already indicated the principal difficulty in answering Dr. Hopps' question: the relationship between these adrenal changes and circulatory collapse cannot be fully understood until the rôle of the several other factors known or thought to be influenced by adrenal cortical function has been elucidated. Some of these are the following: salt and water metabolism, protein and carbohydrate metabolism, possibly control of serum-antibody and blood-lymphocyte levels, as well as maintenance of blood pressure. Changes in all or several of these variables occur simultaneously in the many acute diseases in which these histologic patterns were found, vascular collapse being the only one which is easily recognized clinically. It is certainly true that peripheral circulatory collapse was recorded in a very high percentage of the cases reviewed; and Rich emphasized the correlation between such changes and circulatory collapse in instances of death from overwhelming infection. But he has also pointed out that lesions of this type were not found in cases of death from postoperative shock in the absence of overwhelming infection.

The problem of attributing specific physiologic significance to histologic or cytologic alterations of the adrenal cortex is one that has challenged the efforts of several investigators. The magnitude of this problem becomes apparent when one realizes that adrenal changes occur not only in the many acute diseases described today but also in chronic disease, as for example in the endocrinopathies, hypertension, and possibly some malignancies; also differences of sex, age, and metabolic state, such as puberty, pregnancy, and menopause, may also be associated with recognizable differences in adrenal cortical cyto-architecture.

In order for such physiologic-pathologic correlations to be made, abundant post-mortem or biopsy material must be available from patients who were studied

exhaustively before death. These must be compared with normal controls obtained from violent deaths. Finally, supplemental animal investigations are needed to fill gaps not otherwise bridged. Such studies are in progress.

(Dr. Friedman) In answer to Dr. Boyd's question about unilateral contraction, the other adrenal gland had not been found at autopsy and it was assumed that it had been so markedly contracted that it could not be located.

With regard to syphilis of the adrenal, Dr. Karsner, I will be pleased to have you look at the sections the next time you are in Washington. The patient was being treated with penicillin for a frank taboparesis, serologically proved, and died at the end of the series of treatments, of adrenal insufficiency. At autopsy, lesions showing sclerosing destruction of the parenchyma, considered compatible with syphilis, were found. Only Zenker-fixed tissue was available, so that the Warthin stain could not be done. Possibly we could not have demonstrated spirochetes in view of the treatment.

With regard to Dr. Liebow's question, inclusions have been seen by other workers in some of these lesions. I did not see any, but if viruses can produce comparable lesions in the liver, there is no reason why they cannot in the adrenal.

As far as Dr. Holman's question is concerned, some of these patients gave a history of difficulty following a severe infection. Hemorrhagic lesions, as in the Waterhouse-Friderichsen syndrome, could culminate in contraction, but there has been no evidence that I know of to that effect. I am willing to accept the idea that if the adrenal lesions associated with infection are widespread and severe, and if the patient lives long enough, he might die of Addison's disease due to adrenocortical contraction.

EXPERIMENTAL THIAMINE DEFICIENCY IN THE RHESUS MONKEY. James F. Rinehart and (by invitation) Louis D. Greenberg and Melvin Friedman, San Francisco, Calif.

Abstract. Recent progress in nutrition has made possible the study of single deficiencies in the monkey. It seemed most timely to explore systematically the deficiency states in a primate whose metabolic processes might be expected to approximate most closely those of man. This report is concerned with studies of thiamine deficiency. Seven Rhesus monkeys were subjected to one or more episodes of acute thiamine depletion. It is clear that significant metabolic inadequacies precede demonstrable structural changes. Diminished food consumption and weight loss were manifest about 2 weeks after thiamine was removed from the diet. The blood thiamine at that time was in the range of 4 γ per 100 ml. or less which we believe represents an inadequate content for normal metabolism. As the deficiency is prolonged the animals become apathetic, inactive, and progressively weaker. This is followed by ataxia; at times, ptosis and tremor. Retching was observed in several instances. Even in such advanced states of depletion, administration of thiamine will produce dramatic improvement in locomotion, appetite, and reactivity.

Neuropathologic Findings. The most striking and perhaps most significant lesions were found in the nuclear structures of the central nervous system. While lesions were found in all animals, they were more extensive and severe in those subjected to two or more episodes of depletion. Of the 7 animals studied, bilateral symmetrical foci of degeneration were observed most commonly in the putamen (5), caudate nucleus (4), inferior colliculi (4), cerebellar vermix (4), and certain cranial nerve nuclei. Six of the 7 animals showed involvement of one or more of the 3rd, 6th, 8th, or 10th (dorsal) cranial nerve nuclei. The earliest change was localized edema with separation of glial and nerve fibers and fragmentation and disintegration of myelin sheaths. A process resembling acute ischemic necrosis was seen in some instances. With progression of either type of lesion there was dis-

integration of axis cylinders, degeneration of glia and accumulation of many microglia and fewer astrocytes. The vascular proliferative reactions described in Wernicke's disease were occasionally seen but were not prominent. Structural changes were not demonstrable in the peripheral nerves and no significant lesions were seen in the spinal cord. These observations suggest that thiamine depletion might be a contributory or major factor in some cases of Parkinson's disease.

Blood Formation. Our experiments have shown the regular occurrence of a moderate anemia characterized by a suppression of reticulocytosis.

The Heart. Major interest has revolved about the effect of thiamine deficiency on the heart. Accumulated clinical and experimental evidence leaves little doubt that a functional and structural defect results. We have found the right side of the heart to be dilated, at times appearing as if the muscle were stretched. Histologic examination revealed small foci of myocardial necrosis as previously found in experimental thiamine deficiency in pigeons, rats, and pigs. Another lesion was a well defined hydropic degeneration of myocardial fibers with hyperplastic nuclear changes involving, particularly, subendocardial fibers, presumably of the conduction system. This lesion is like that described by Wenckebach in human deficiency.

Discussion

(Dr. Richard H. Follis, Jr., Baltimore, Md.) I should like to ask an obvious, but important question; that is, what supplements were given, and in what form were they given?

(Dr. John H. Fisher, London, Ont.) The cerebral lesions, which Dr. Rinehart described, are highly comparable to those seen in arsphenamine encephalopathy, particularly as to the character of the lesions, and their peculiar symmetrical distribution. I think it has been claimed recently that the lesions seen in arsphenamine encephalopathy are perhaps due to thiamine deficiency rather than to any actual direct toxic effect of the arsphenamine itself. I have observed a fatal case of arsphenamine intoxication in which, at autopsy, the brain showed multiple symmetrical foci of hemorrhagic necrosis, characteristic of arsphenamine encephalopathy. During the course of treatment the thiamine level in this patient's blood was greatly lowered.

(Dr. William Boyd, Toronto, Ont.) I should like to ask Dr. Rinehart if he has any comment to make on the possible relationship of these experimental lesions to those cases which Dock and his associates described of myocardial failure attributed to deficiency of thiamine or vitamin B complex. These cases were characterized by two pathologic changes. One was the remarkable increase in the weight of the heart; the average weight of the heart in the five or more cases was about 650 gm. The second point was a remarkable subendocardial fibrosis. I should be particularly interested to hear the distribution of the myocardial fibrosis in Dr. Rinehart's animals.

(Dr. Ralph D. Lillie, Washington, D.C.) Some 20 years ago when we were first separating B1 from the vitamin B complex, I had a series of some of the first rats in which a rather prolonged polyneuritis had been produced, and had the enjoyable experience of looking over their brain sections and found absolutely nothing to account for this. I am very much interested in Dr. Rinehart's success in demonstrating intracerebral lesions.

(Dr. Helen Ingleby, Philadelphia, Pa.) The myocardial changes shown are very similar to those of the human heart in beriberi.

(Dr. Alfred Angrist, Jamaica, N.Y.) In my experience, the myocardial changes noted occur quite commonly in routine human material, and we have always considered them as evidence of circulatory insufficiency (anoxic) of rather marked degree. I am reasonably certain that they will be found in the human being in cases in which no gross dietary deficiency can be demonstrated in the history.

(Dr. Rinehart) With regard to the question of Dr. Follis, the diet was essentially that used by Waisman, and is essentially a synthetic diet. The diet was fed to the animals in tablets.

In reply to Dr. Boyd's question, I think this does bear some resemblance to the condition of which Dr. Dock spoke. If it were a very prolonged experiment one might get subendocardial fibrosis of some degree. Most of the animals were subjected to two or more episodes of acute depletion. No doubt the pathologic changes were accentuated by this procedure.

In regard to Dr. Fisher's comment, I am much interested in his reference to arsphenamine. Possibly this inhibits the enzyme mechanism involving thiamine.

Thrombi were not found in the brain or in the heart muscle. Frankly I do not understand the pathogenesis of the lesions. I undertook the work in part to try to satisfy myself about the heart changes in thiamine deficiency. The literature is just a little confusing, and I thought with this experimenal background I would have a little better chance to recognize it in the human being. I would, however, hesitate to diagnose this cardiac lesion on histologic grounds alone. I would like to have confirmation of biochemical observations.

SYMPOSIUM ON HEPATIC INJURY

NECROTIZING HEPATIC INJURY AND ITS SEQUELS.* Balduin Lucké, Philadelphia, Pa.

LIVER NECROSIS PRODUCED WITH SODIUM TANNATE. F. W. Hartman, Detroit, Mich.

Abstract. Alkaline sodium tannate, pH 10, may be slowly injected intravenously with little or no immediate reaction on the part of the experimental animal. The solution of sodium tannate is made by dissolving 7.5 gm. of U.S.P. tannic acid in 100 cc. of distilled water and adding 10 per cent sodium hydroxide until a pH of 10 is obtained. Injection intravenously of 2 to 3 cc. of this solution per kg. in the dog or rabbit results usually in rapidly developing jaundice, loss of appetite, loss of weight, coma, and death. Autopsy shows all tissue icteric and the liver either large and congested, or small, flabby, and greenish yellow. The microscopic examination of the liver in most instances reveals a central necrosis of varying extent.

FATTY INFILTRATION, NECROSIS, AND SCARRING OF THE LIVER PRODUCED BY DIETARY MEANS AND MODIFIED BY THYROID ACTIVITY. Richard H. Follis, Jr.† and (by invitation) Philip Handler, Durham, N.C.

Abstract. The production of hepatic lesions by dietary means is now well recognized. When rats are placed on synthetic rations of low protein content with added cystine and containing varying amounts of fat, but no choline, extreme fatty infiltration of the liver is found. In time certain hepatic cells become necrotic and are replaced by scars. In some livers an acid-fast, fluorescent pigment, ceroid, appears; this is related to the cod-liver oil content of the diet. When other animals are placed on low protein diets without added cystine, but with supplements of choline, acute and widespread necrosis is found; such animals usually die rather suddenly. The lesions observed in choline or cystine deficiency are therefore different. Our observations have dealt with the modification of these lesions by thyroid activity, since we have noted differences in the hepatic lesions of animals whose choline-deficient diets contained sulfasuxidine. It will be recalled that

* By invitation of the Council.

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Gyorgy and Goldblatt have reported a protective effect when thiouracil is added to a choline-deficient regimen.

A. When anti-thyroid substances (sulfasuxidine, thiouracil, and para-amino-benzoic acid) are administered, the histological picture ordinarily produced by choline deficiency is changed. Such anti-thyroid substances lead to an accentuation of the fatty infiltration in the liver, yet seem to delay the production of necrosis and scarring; protection is by no means complete, however.

B. When thyroid extract is administered to choline-deficient animals, there is a reduction in the fatty infiltration. However, since such animals die relatively early, its effects on necrosis and scarring have not been determined as yet.

C. The effects of thyroid activity on cystine-deficient rats are equivocal. Added thyroid extract does not seem to retard or accelerate necrosis in any degree. We have not studied the effects of anti-thyroid substances on a cystine-deficient regimen.

D. As is well known, sulfasuxidine, para-amino-benzoic acid, and the thiouracils lead to anatomic thyroid hyperplasia but physiologic thyroid hypofunction. Thyroid extract leads to anatomic thyroid hypoplasia and physiologic glandular hypofunction. It is of some interest, therefore, that choline deficiency leads to anatomic thyroid hyperplasia. Whether there is an accompanying hyperfunction, eufunction, or hypofunction remains to be determined. It is of interest, however, that this observation is compatible with morphologic and chemical data available on the relationships between thyroid function and liver fat.

E. Raising the fat content of the diet increases the fat in the liver of choline-deficient rats and tends to accentuate the necrosis of cystine deficiency.

UNANTICIPATED LIVER CHANGES IN DOGS FOLLOWING CESSATION OF A HIGH-FAT DIET. Russell L. Holman, New Orleans, La.

Abstract. During experiments which involved feeding dogs a specified high-fat diet for 2 months or longer, followed by an interval of 1 to 4 weeks of kennel diet without fat supplement, then sacrifice with induced renal insufficiency (uranium nitrate), marked increases in liver weight associated with occasional histologic changes in the liver were noted. When these experiments were controlled by using a similar period of high-fat feeding, followed by similar intervals of kennel diet without fat supplement, but instead of heavy metal injury the dogs were sacrificed with a blow on the head, the increases in liver weight did not occur, but the histologic changes were more marked. All 4 dogs subjected to this latter procedure showed atypical focal necrosis in the liver and in one of these there were, in addition, early cirrhotic changes. In a large number of control dogs no such changes have been observed. These unanticipated findings, which obviously need confirmation and extension, suggest that simple withdrawal from a high-fat diet may not be without some dangers. They further suggest that fat absorbed from the gastrointestinal tract may pass through the liver with greater ease than fat resorbed from the depots.

HEPATIC NECROSIS INCIDENT TO SHOCK. Virgil H. Moon, Philadelphia, Pa.

Abstract. Hepatic degeneration and necrosis occur in secondary shock resulting from wounds, burns, intoxications, infections, poisons, heat stroke, and from other causes. Corresponding changes are found after experimental shock produced by similar agents. The necroses vary in amount and in location. Scattered groups of necrotic cells occur in central, mid-zonal, or in peripheral areas of the lobules; often necrosis is most marked in the central area. In exceptional cases, as after extensive burns, heat strokes, or severe intoxication, the liver resembles "acute yellow atrophy." The cause of these hepatic effects is obscure. Toxic substances from burned or infected areas have been suggested. However, the same effects

are seen after shock from lack of oxygen, heat stroke, anaphylaxis, and from low barometric pressure as in high-altitude aviation, in which instance no source for toxic substances is apparent. Anoxia is a common denominator in shock from diverse causes; perhaps degeneration and necrosis result from anoxia.

A Cytochemical Study of Regenerating Rat Liver. Robert E. Stowell, St. Louis, Mo.

Abstract. Following the removal of 60 per cent of the liver of adult rats by partial hepatectomy, the regenerating liver was studied at frequent time intervals up to 2 weeks. Sections of surgically removed (control) tissue and of regenerating liver which were frozen-dried by the Altman-Gersh method or fixed in Stieve or Carnoy fluid were (1) photographed in ultraviolet light of 2750 Å, or (2) stained with hematoxylin and eosin, or (3) with the Feulgen reaction for thymonucleic acid. Six hours after partial hepatectomy, vacuolization of the cytoplasm of the hepatic cells was evident and the cells continued to increase in volume up to the time of increased mitotic division. The mean size of the nucleoli, which by 24 hours had increased 3½ times, returned to normal by 48 hours. The number of nucleoli per nuclear section increased during the second day. The increased light absorption in regenerating liver at 2570 Å, which is characteristic of nucleic acid, was confirmed by macrochemical analysis showing a significant increase in total phosphorus and in percentage of nucleotides of the ribose type. The results illustrate the relationship of the nucleolus, ribose nucleic acid, and protein metabolism of hepatic cells.

Discussion

(Dr. Alfred Angrist, Jamaica, N.Y.) This work of Dr. Stowell has been of considerable interest to us, and we find it has a distinct application to the subject of our symposium.

We have been studying nucleic acid partitions in regenerating liver. Our work was initiated by finding that in ordinary old and advanced Laënnec's cirrhosis, in which the morphologic pathologist feels that the liver tissue is quite static, we got figures by chemical analysis such as obtain for a more youthful type of cell than the normal, approaching data we get in embryonic tissue or in a tissue that is rapidly regenerating. The proportion of ribose nucleic acid to desoxynucleic acid was determined by using the fractionating method of getting different proportions extractable at different temperatures, which is the technic used in ordinary polymer research (and I think that everybody is agreed that nucleic acid exists in the body in polymeric form). I repeat that our results, suggesting a shorter polymeric chain structure of the nucleic acid moiety, hold true for ordinary cirrhosis.

We carried the work further to attempt observations on "butter yellow" livers, in which obvious regeneration occurs. When we tried to use the large tumorous nodules for chemical analysis, with the remaining nontumor-bearing liver of such animals as a comparison for control, we found that the apparently normal liver in "butter yellow" animals was unsuited as normal control material because it showed intermediate values between those for the true hepatomas or cholangiomas of the "butter-yellow" livers and those obtained from livers of rats on normal diets without the "butter-yellow." This indicated a regenerative process not obvious morphologically, in keeping with Dr. Stowell's findings without any butter-yellow.

The significance of these findings, I think, is to be found in the insight it gives us into the biochemical significance of the nucleoproteins in relation to cellular regeneration. Our initial work, which will be reported later this week before the Society for Experimental Biology and Medicine, seems to indicate that it is not only the total quantity of the nucleic acid, which Dr. Stowell has presented, but also the state of the nucleic acids, as far as their chain formations, in long or

short chains of varying length for each of the molecules, which is important. This may be a little obtuse at the moment, but it surely bears further study.

EPIDEMIC HEPATITIS WITH RUPTURE OF THE SPLEEN IN THE PRE-ICTERIC PHASE. A REPORT OF A CASE. John L. Work and (by invitation) H. Garrett Van der Veer, Montclair, N.J.

Abstract. A previously healthy 27-year-old white man was operated upon 4 days after the onset of chills, fever, nausea, vomiting, and abdominal cramps. The laparotomy revealed hemoperitoneum due to rupture of the spleen, and a splenectomy was performed. Jaundice developed on the third postoperative day, and the patient died 5 days later. The spleen was slightly enlarged and microscopic sections revealed severe hyperplasia of the pulp. The most important abnormalities disclosed by the autopsy were in the liver. The changes were those which have been described as characteristic of epidemic hepatitis. There was also right-sided dilatation of the heart, bilateral bronchopneumonia, edema of the esophagus, stomach, and intestines, acute enteritis and colitis, and ascites.

SEQUEL TO NECROTIZING HEPATITIS. Max M. Strumia, Bryn Mawr, Pa.

Abstract. A 43-year-old male was first admitted to the hospital in September, 1945, because of compression fractures of the 4th and 5th dorsal vertebrae, fractured clavicle, and cerebral concussion following an automobile accident. The patient's history revealed jaundice 20 years prior to admission, lasting 2 weeks. The patient remained in a body cast for 3 months, during which time he ate poorly and lost considerable weight. He did not receive blood, plasma, or serum. In April, 1946, slight jaundice was noted, which became progressively worse. He was readmitted on May 30, 1946, with pitting edema of the ankles, enlarged liver, and palpable spleen. Laboratory studies showed a serum bilirubin of 14 mg.; cholesterol, 113 mg.; cholesterol esters, 26 per cent; phosphatase, 6.2 Bodansky units; total protein, 4.6 gm. per 100 cc.; albumin, 2.9 per cent; prothrombin, 45 per cent of normal; urea nitrogen, 5 mg. per 100 cc.; nonprotein nitrogen, 44 mg. per 100 cc.; and markedly increased bromsulfalein retention. He was treated with high-protein, low-fat, high-carbohydrate diet and with protein feedings intravenously. Peritoneoscopy revealed a cirrhotic liver. Four thousand cc. of ascitic fluid were removed. The patient improved considerably and was discharged on June 14, 1946. He did fairly well at home until the morning of June 24, when he had a sudden attack of coughing, followed by vomiting bright red blood. He rapidly became unconscious and died shortly after. The autopsy revealed remnants of a necrotizing lesion typical of infectious hepatitis, a typical post-necrotic cirrhosis. It is felt that without the esophageal hemorrhage this patient might have survived with development of a full-fledged cirrhotic lesion.

CIRRHOSIS FOLLOWING INFECTIOUS HEPATITIS. Walter H. Sheldon and (by invitation) David F. James, Atlanta, Ga.

Abstract. Infectious hepatitis, after the subsidence of acute symptoms, often leaves impaired liver function. This has been substantiated by many clinical but relatively few morphologic observations. The latter have created confusion as to the type of lesion resulting, which has been classified as Laënnec's cirrhosis, "nodular" cirrhosis, or as "cholangiolitic" cirrhosis. We have studied 3 cases in which we believe infectious hepatitis ended in a lesion described by F. B. Mallory as "toxic" cirrhosis. Two of our patients had bouts of hepatitis 5 years before their final admission. Interim studies available in one showed persistence of impaired hepatic function. The clinical picture on final admission of all 3 patients was that of hepatic failure with portal hypertension.

The autopsy findings in the 3 patients were similar. The livers were small and

irregularly nodular. The tissue between the nodules was shrunken and atrophic. The cut surfaces showed complete loss of normal architecture. On histologic examination the nodules consisted of regenerated liver cells separated by wide areas of stroma which was collapsed and devoid of parenchyma. The liver cells in many nodules displayed slowly progressive degenerative changes of varying, often considerable, extent, ranging from fatty metamorphosis to necrosis. The central and midzonal portions were chiefly involved, while the peripheral cells showed regeneration. The stroma in the areas devoid of liver cells showed preservation of the lobular pattern, without new formation of either reticulum or collagen. Similarly, the portal spaces revealed only condensation of the stroma without scarring. A mild chronic inflammatory infiltration was present. Bile casts were frequent in the intercellular bile canaliculi. The perilobular bile ducts were increased in number, while the larger ducts were not remarkable. Other organs showed the effects of portal hypertension, with splenomegaly and œsophageal varices.

The active degenerative changes indicated the progressive character of the lesion, which otherwise was similar to subacute infectious hepatitis. These changes may be secondary to ischemia and accumulated metabolic products, but one might speculate that persistence of the viral infection could account for this picture. Our findings are identical with the lesion described variously as "toxic" or "post-necrotic" cirrhosis and as "healed acute yellow atrophy." We conclude that infectious hepatitis sometimes results in chronic progressive liver disease identical with "toxic" or "post-necrotic" cirrhosis. The lesion can be distinguished from other types of cirrhosis.

INFECTIOUS VS. TOXIC HEPATITIS IN THE CIVILIAN POPULATION. Hans Popper and (by invitation) Murray Franklin, Chicago, Ill.

Abstract. This paper is based on a study of all cases of fatal hepatitis observed at Cook County Hospital in the past 18 years. The differences are discussed which exist between the infectious (viral) form, which is similar to the great majority of the cases observed in military personnel, and the different forms of toxic hepatitis which represent the majority of the cases in our material. The criteria available in post-mortem material have been applied to a series of liver specimens obtained for biopsy by needle from cases of primary hepatitis. As a result, in the majority of these patients a differentiation into toxic and infectious groups was possible.

XANTHOMATOUS BILIARY CIRRHOSIS.* H. Edward MacMahon, Boston, Mass.

Abstract. A histologic study of adequate "surgical biopsies" from the livers of 4 patients considered by Thannhauser as classical examples of a syndrome which he called "xanthomatous biliary cirrhosis" revealed a chronic inflammatory reaction throughout the portal areas. This reaction was most concentrated about the terminal and junctional bile ducts. There was a proliferation of inflammatory granulation tissue, a moderate cellular infiltration, a disappearance of terminal bile ducts, degeneration and necrosis of liver cells at the periphery of the lobules, regeneration of liver cells with formation of new junctional ducts, and, finally, moderately severe intralobular bile stasis. At times the inflammatory reaction cut deeply into the lobules and isolated islands of liver cells. For the most part, the lobular pattern and the central veins, central zones and mid-zones were well preserved. No xanthoma cells were found in any of the sections. No bile or leukocytes were seen in any of the bile ducts. No organisms were demonstrable. The picture was one of chronic pericholangiolitis with early and uniform perilobular fibrosis. It had features in common with both obstructive and cholangiolitic cirrhosis, but it was distinct from each of them.

* This article will appear in a subsequent issue of *The American Journal of Pathology.*

Because the anticipated lesion of xanthomatous biliary cirrhosis of the liver could not be substantiated in sections, it is suggested that this name as applied to cirrhosis should be discontinued. To indicate the original site of the inflammatory reaction, to denote the inflammatory nature of the lesion, and to indicate the ultimate fate of the liver, the term "pericholangiolitic biliary cirrhosis" is suggested to indicate the type of disease found in the four cases examined.

Discussion

(Dr. Paul Kimmelstiel, Charlotte, N.C.) In view of the rarity of this condition, it may be of interest to mention a case of so-called xanthomatous cirrhosis which we observed several years ago. The case was that of a middle-aged woman with widespread xanthomatosis of the skin, extremely high cholesterol level of the blood, and gradually increasing jaundice and ascites, fulfilling all of Thannhauser's criteria. The post-mortem examination showed an ordinary Laënnec's cirrhosis of the liver. No xanthomatous lesions were found in extrahepatic or intrahepatic bile ducts and the histologic sections did not suggest biliary cirrhosis. I am inclined to agree with Dr. MacMahon that it is questionable whether the concept of xanthomatous cirrhosis as a disease entity is worth being maintained.

(Dr. Howard T. Karsner, Cleveland, Ohio) There are two questions I would like to put to Dr. MacMahon. The first has to do with the designation. As I have seen the lantern slides, I wonder if at the beginning this is not what Rössle called cholangitic, and subsequently what may have become, and probably did become, cholangiolitic. The other question is this: In talking with several of my clinical colleagues about cases in which they have made the diagnosis of xanthomatous cirrhosis, I have found that in each instance (the number is very small, 3 or 4), jaundice preceded the development of xanthomatosis by several months. That raises the question, then, as to whether the relationship is xanthomatosis-cirrhosis, or cirrhosis-xanthomatosis.

(Dr. MacMahon) In answer to Dr. Karsner's question in regard to whether this is cholangitic cirrhosis, as the term was employed by Professor Rössle: It was my good fortune to have spent a number of years with the late Professor Mallory, and to have familiarized myself with the lesion which he described as "infectious cirrhosis." This was an infection of the small bile ducts and was considered by him to be bacterial in origin. I also had the opportunity to work on the liver under Professor Rössle in Berlin. At that time I showed him slides which Dr. Mallory had called "infectious cirrhosis" and these he called cholangitic cirrhosis. The lesion in the liver which I have presented today and called "pericholangiolitic biliary cirrhosis" simulates the cholangitic cirrhosis of Rössle (infectious cirrhosis of Mallory) but may be distinguished from it. The characteristic features of cholangitic cirrhosis (Rössle) are an inflammatory exudate within the lumina of the interlobular bile ducts, a proliferation of these ducts, and an unevenness in the extent and distribution of the inflammatory lesion. In pericholangiolitic biliary cirrhosis during an equally active phase, there are no leukocytes in the bile ducts, the inflammatory reaction is concentrated about the cholangioles, there is a diminution or complete loss of small bile ducts, and the lesion is strikingly uniform in distribution. Under low magnification, these two diseases have much in common, but I believe it is possible, particularly in the early phase, to distinguish one from the other. At this point, it must be admitted that the lesions in pericholangiolitic biliary cirrhosis resemble more closely a type of biliary cirrhosis described by Rössle under the name of "cholangiolotische Zirrhose" (cholangiotoxische Zirrhose). Because no toxin has been definitely identified in association with the lesions which I have just described, the purely descriptive term of "pericholangiolitic biliary cirrhosis" has been chosen.

In these 4 cases and in all cases that have been reported in the literature as show-

ing this clinical syndrome, jaundice has preceded xanthomatosis by at least 6 months, and in some cases by as long as 2 years. It was an interesting finding that of all the cases recorded, 87 per cent have been in females, irrespective of the underlying changes in the liver. The average age was about 40, and it is perhaps worth mentioning that for many months after the appearance of jaundice the great majority of these patients have remained remarkably well.

POSSIBLE RELATION OF DUODENITIS AND DUODENAL ULCER TO HEPATIC CIRRHOSIS. William Carpenter MacCarty, Sr., Rochester, Minn.

Abstract. In the symposium of twelve papers of which this is a part, I am glad to see that the term cirrhosis has been used only three times in the titles. Fortunately, and perhaps correctly, we are beginning to speak of chronic hepatitis, an expression which includes the late stages described long before we had opportunities to study the early stages at surgical exploration. Among the many causes of advanced chronic hepatitis, one rarely hears of duodenitis and duodenal ulcers and their possible relation to this condition. As long ago as 1872, 1876, 1882, and 1908, Meyer, Charcot and Gombault, Maffucci and Tsunoda described, respectively, the experimental production of biliary cirrhosis by artificial stenosis and partial stenosis of the common duct, which is not an uncommon surgical condition produced by duodenal ulcer. Formerly it was thought that most duodenal ulcers were single and near the pylorus; duodenal ulcers are, however, very often multiple and exist just above, at, and just below the papilla of Vater. In these portions of the duodenum it is quite reasonable to think of repeated interference with the patency of both the common bile and pancreatic ducts, thereby producing unfavorable changes in both the liver and the pancreas. From a large series I have chosen two examples of the association of cirrhosis with duodenal ulcers which partially obstructed the common duct.

Case 1. A male, aged 60 years, was operated upon for cholecystitis 17 months before the last operation. He improved for 5 or 6 months and again had epigastric pains, jaundice, and vomiting. At the second operation the gallbladder was distended, the common duct dilated, and the head of the pancreas very hard. The patient died on the 12th day following the re-operation and the necropsy showed a marked chronic hepatitis (called cirrhosis), chronic cholecystitis, chronic pancreatitis, chronic congestion of the spleen, and a chronic ulcer of the duodenum at the papilla of Vater about 1 cm. in diameter. There was almost complete stenosis of the common duct at the base of the ulcer.

Case 2. A male, aged 58, 4 years before examination had had attacks of severe epigastric pain which radiated to the back. Seven years before the last operation he had similar attacks. At operation one gallstone was removed and the gallbladder was drained. There was a hard mass felt at the end of the common duct, thought clinically to be a carcinoma. The patient died on the 4th postoperative day from a hemorrhage into the bowel. At post-mortem examination there was a duodenal ulcer 2 cm. in diameter with the common duct running through the base of the ulcer. There was a very definite biliary hepatitis, called cirrhosis. The surgeon noted that the liver was "angiomatous."

My purpose is merely to stimulate others to watch for this association at autopsy and at surgical exploration. Even experimental production of partial or periodic occlusion of the common duct and pancreatic duct might throw light on some of the diseases of the liver and pancreas which have been considered primary entities rather than sequelae of duodenal pathologic conditions.

Discussion

(Dr. Max M. Strumia, Bryn Mawr, Pa., replying to a comment by Dr. MacCarty) In my abstract of the case which I reported today, I stated that the

stomach contained 400 cc. of clotted blood; the small intestine was filled with tarry material, presumably blood. The mucosa of the stomach was flattened. There was a break in one esophageal vein which measured 3 mm. The mucosa of the duodenum was thickened and red. Most of the mucosa of the cecum was pale and showed no gross lesions. The remainder of the gastrointestinal tract appeared normal. Histologic examination showed that the duodenum was congested. The colon seemed to show a mild chronic colitis.

INJURIES PRODUCED BY THE ATOMIC BOMB

OBSERVATIONS ON HUMAN BEINGS AT HIROSHIMA AND NAGASAKI

MECHANICAL INJURIES AND BURNS. Averill A. Liebow and Shields Warren, New Haven, Conn., and Boston, Mass.

Abstract. Direct blast injury analogous to that inflicted by high explosives was almost unknown among survivors at Hiroshima and Nagasaki as indicated by an incidence of ruptured eardrums of about 1 per cent. Almost universal, however, were injuries produced by flying glass and the falling beams of wooden houses. The more severe injuries were rare since those that had been severely injured were killed by fires that swept the city before rescue operations could be instituted. The burns among survivors were largely of the "flash" type, the result of an exceedingly large quantity of radiant heat acting for an exceedingly brief interval. Only survivors in the rectilinear path of the rays were involved, so that the burns were of a sharply outlined "profile" or "mask" type. Depigmentation at the center with marginal hyperpigmentation of the burns was prominent in patients close to the bomb, but at greater distances the entire exposed surface became intensely pigmented, and the pigment showed no tendency to fade within 4 months. There was histologic evidence that the depigmentation occurred without destruction of the squamous epithelium of the surface, suggesting the action of specific wave lengths. Even minor injuries and burns became serious foci of infection in persons who also suffered the leukopenia resulting from radiation.

EARLY EFFECTS OF RADIATION. Averill A. Liebow and Shields Warren, New Haven, Conn., and Boston, Mass.

Abstract. The effects of the ionizing radiations resembled closely those produced by x-rays in animals and men. Nausea and vomiting occurred in many adequately exposed persons within a few hours after the bomb. A special effort was made to center the study of the radiation effects upon tissues from patients who had sustained little or no other injury. The earliest autopsy material is from persons dying "mysteriously" with symptoms of severe diarrhea and fever on the third day after the bomb. In them, epilation and the clinical manifestations of aplastic anemia (except leukopenia) had not had time to appear.

The Skin. In a few cases there were epithelial changes at the margins of ulcerative lesions in patients dying in the third week, but since most persons who received as much as an erythema dose over the whole body died during the first confused days when autopsies were extremely rare, little material is available for study. Epilation in both men and women began 14 to 20 days after the bomb. It involved chiefly the scalp in a distribution resembling that of ordinary baldness. Histologically, the mechanism is entirely analogous to that of the usual processes of loss and replacement of the hair, with arrest of mitosis in the matrix, failure of differentiation of the internal root sheath as the old hair is extruded, and finally (some 2 months after the bombing) evidences of renewed differentiation of the internal root sheath with penetration of the new hair through the old external sheath to the surface.

Gastrointestinal Tract. Typical radiation changes were seen in the intestines of

persons dying as early as the fourth day. These consisted of bizarre cells, some with enormous nuclei possessing a coarse chromatin network and a large body of cytoplasm. Atypical mitotic figures were found in some cells and tripolar mitotic figures were observed. In one patient dying on the tenth day, the cytoplasm and nuclei of the squamous epithelial cells were remarkably swollen, and fragmentation of the nuclei was observed.

Gonads. Even at the fourth day remarkable changes were found in the testes, with sloughing of the germinal epithelium, together with an increase in Sertoli cells. Toward the end of the first month, there was almost complete loss of germinal epithelium. After the fifth week the tubules began to display thickening of the basement membrane and there were hyaline deposits restricting the lumina of the interstitial blood vessels. There was slight hyperplasia of the interstitial tissue after the end of the sixth week. To correlate with the changes in the testes, there was a remarkable decrease in the count of the spermatozoa of patients who had been close to the bomb. How permanent this will be is at the present time unknown. Occasionally, "castration cells" were found in the pituitary glands.

Much less striking changes were observed in the ovary. A few primary follicles were in process of atresia. The most usual finding was that of the absence of developing follicles despite the persistence of primary follicles. The endometrium showed an absence of corpus luteum effect.

Lymphoid Tissues. Even after 3 days there was a remarkable degree of atrophy of the lymphoid tissues, leaving nothing but the reticular skeleton. Beginning on the fifth day, however, large numbers of atypical mononuclear cells resembling lymphoblasts or Reed-Sternberg cells began to appear. These gradually decreased in number during the following 3 months and in a few instances secondary follicles had reappeared by this time both in the spleen and lymph nodes.

Bone Marrow. Even within the first 4 days there was, in severely exposed individuals, almost total loss of all myeloid and erythroid tissue, but there was evidence of proliferative activity, with new reticulum cells, often as bizarre as those seen in the lymph nodes, making their appearance. During the first month such proliferative activity became remarkable in many cases, but the products were largely atypical reticulum cells and plasma cells. In some patients, at variable intervals, there was renewed differentiation into granulopoietic and erythropoietic tissue, and in some, who died toward the end of the sixth week, there was actually hyperplasia of these cells, although peripheral leukopenia had been noted.

PATHOLOGIC ANATOMY OF LETHAL IONIZING RADIATION: LATE DEVELOPMENTS. Elbert DeCoursey, Fort Sam Houston, Tex.

Abstract. The prominent lesions are presented which are seen in human beings dying from 7 to 15 weeks after instantaneous ionizing radiation from an atomic explosion. Not as many individuals of this group had had nausea and vomiting on the first day or as severe leukopenia, purpura, or oropharyngeal lesions as had those dying earlier. Emaciation is the usual finding. The scalp, femoral bone marrow, spleen, and intestines have striking gross changes, and the bone marrow, lymph nodes, and testes have distinctive microscopic changes.

In the skin, hemorrhagic lesions become uncommon, hair follicles begin to regenerate, decubital ulcers are usually present, and most of the flash burns are healed, but some, still granulating, appear keloidal.

The femoral bone marrow is usually pink in the upper third or half and all marrow shows more and more myeloid hyperplasia but usually with some maturation defect. Severe anemia has usually been present before death.

The spleen and lymph nodes remain atrophic.

The testes are more atrophic and contain almost no spermatogenic cells, the Leydig cells being apparently uninvolved.

The adrenals are uniformly small and lipoid-deficient.

The gastrointestinal tract presents the most striking changes, which vary from petechiae to widespread pseudomembranous ulcerative involvement resembling bacillary dysentery. These lesions and necrotizing pneumonia are the leading causes of death. The heart, thyroid, salivary glands, brain, liver, pancreas, prostate, kidneys, and bladder show no constant radiation-connected changes except for occasional petechiae or a rare bacterial clump. There is no effect on growing cartilage or bone.

PATHOLOGY OF THE EYE IN ATOMIC BOMB CASUALTIES. Helenor Campbell Wilder (by invitation), Washington, D.C.

Abstract. The following observations were based on the microscopic examination of 18 eyes removed at autopsy from Japanese who died 24 to 33 days after the Hiroshima bombing. In no instance was an entire globe or both eyes of a patient received.

Breaks in Bowman's membrane were seen in 4 eyes and may have been the result of direct injury. They also occur as senile phenomena. In 6 eyes there was elastosis of the conjunctival stroma. This, however, is an almost constant finding in persons over 50 years of age and may occur in young people as a result of exposure.

In one instance there was hemorrhage in the conjunctiva; in another, a small pre-retinal hemorrhage with a few red cells streaming out into the vitreous chamber; and in a third, choroidal hemorrhage. In one retina there were small, apparently edematous areas in the nerve fiber layer. In these the nerve fibers appeared swollen, and occasional homogeneous structures were somewhat suggestive of the cytoid bodies one sees in hypertensive vascular disease with renal retinopathy, and in choked disk. Although this patient was only 23 years old, the lesions must be considered as possibly related to central serous chorioretinitis which is common in Japanese, but affecting, particularly, men of middle age. They probably correspond to the white spots in the fundus observed by Flick.

Septic choroiditis was present in 17 of the 18 eyes. It was manifested by infiltration, particularly of the posterior choroid, by large mononuclear cells occasionally in mitosis, and a scattering of lymphocytes, plasma cells, and Russell bodies. Large mononuclear cells frequently packed choroidal veins, but were rarely found in the arteries. In one case bacilli resembling those found in the bone marrow of the same patient engorged many of the capillaries of the choriocapillaris.

Degenerative changes were seen in the lens in 15 eyes; in 2 eyes the lens was not included in the blocks, and in one it appeared normal. The changes were limited to the cortical fibers and were more prominent at the posterior pole. Here the fibers were swollen and pale-staining. Vacuoles of various sizes appeared immediately beneath the capsule, which was wrinkled over the larger ones. Anteriorly and at the equator the changes were usually less marked, the vacuoles smaller, and the overlying subcapsular epithelium intact.

Of the pathologic changes in these eyes, cataract alone can be regarded with any degree of plausibility as the direct effect of irradiation. Blood dyscrasia, in itself an effect of irradiation, was the probable cause of the hemorrhages and a possible cause of the retinal edema; septic choroiditis was associated with the consequent severe infections accompanied by septicemia. Breaks in Bowman's membrane and elastosis of the conjunctiva were of doubtful origin and may even have been unrelated to the explosion.

HEMATOLOGY. George V. LeRoy (by invitation), Chicago, Ill.

Abstract. The disturbances in the blood and the blood-forming organs of the Japanese exposed to the atomic bomb were of a type that could be anticipated on

the basis of experimental studies of animals exposed to large single doses of ionizing radiations. The most important observations that were made in Japan concerned the rate at whch aplastic anemia developed, and the character and tempo of the recovery process, when it occurred. In the most heavily irradiated individuals the syndrome was fully developed and death occurred within a period of approximately 2 weeks. Even in such patients, however, there was evidence of some proliferation of the reticulo-endothelium of the hemopoietic system. In less heavily irradiated individuals the maximum depression of the activity of the blood-forming organs occurred between the 4th and the 6th week after exposure. The leukocyte count, as an average, was lowest during the 4th and 5th week, and had returned to normal levels between the 8th and 9th week after the bombing. The erythrocyte count and the thrombocyte count reached the lowest levels during the 6th to the 8th week, and returned to normal values at about the 12th to the 16th week after the bombing. The tendency of the bone marrow to regenerate was observable in all specimens studied. In some patients it appeared that regeneration proceeded from normal hemopoietic elements that had survived irradiation. In others, proliferation of the reticulo-endothelium with the evolution of functional marrow from the products of this system was observed. In some preparations there was evidence of the development of neutrophilic myelocytes directly from the reticulo-endothelium without the appearance of recognizable myeloblast-like forms. The clinical significance of the tendency for heavily irradiated bone marrow with aplastic anemia to recover is of obvious importance in the management of irradiated patients.

OBSERVATIONS ON ANIMALS EXPOSED AT BIKINI

MECHANICAL INJURIES AND BURNS IN THE BIKINI ANIMALS. R. Harold Draeger (by invitation), Washington, D.C.

Abstract. In Operation Crossroads, goats, pigs, and rats were exposed on target ships at Bikini during Tests Able and Baker in order to determine the probable effects of an atomic bomb explosion upon naval personnel. About 30 per cent of the animals died from the effects of the bombs. The majority of these deaths were caused by ionizing radiation; about 10 per cent of the fatalities were due to air blast. Flashburn was not an important factor since the fur of the animals in most instances provided efficient protection.

THE PATHOLOGIC CHANGES INDUCED BY IONIZING RADIATIONS IN THE BIKINI ANIMALS. John L. Tullis (by invitation), Washington, D.C.

Abstract. The animals which received a fatal dose of ionizing radiation during the atomic bomb tests at Bikini died within 1 month after exposure. There have been no deaths due to latent or chronic radiation injury. The gross pathologic lesions were, in general, of three types: (1) widespread hemorrhage throughout the organs of the body, causing severe anemia; (2) secondary infections, usually involving only the lungs; (3) degenerative changes, manifest chiefly as ulcerations of the gastrointestinal tract and tonsillar tissue.

THE HEMATOLOGY OF THE BIKINI ANIMALS. Eugene P. Cronkite (by invitation), Washington, D.C.

Abstract. In Bikini Test Baker, animals were exposed to massive fatal doses of ionizing radiations over a period of 4 to 5 days. These were of two magnitudes: the greater dose resulted in a marked leukopenia involving all cells and slight hemorrhagic phenomena at a time when platelets were present and the clotting time was normal; the lesser dose resulted also in similar changes in the leukocytes and in a severe hemorrhagic diathesis that may be divided into three stages. In the early stage there was increased capillary fragility with scattered petechiae. In the

second stage there was severe thrombocytopenia accompanied by purpura, while in the terminal stage the clotting time was prolonged. These changes resulted in an extensive purpura with both subcutaneous and subfascial hemorrhages. In the terminal stage the clinical and laboratory findings resembled those in purpura haemorrhagica and hemophilia.

Discussion of Papers on Injuries Produced by the Atomic Bomb

(Dr. Virgil H. Cornell, Washington, D.C.) I would like to ask if any experiments have been conducted with irradiated foods in otherwise unexposed animals, and, if so, whether gastrointestinal lesions were seen in such animals.

(Dr. Sol Roy Rosenthal, Chicago, Ill.) The marked tissue destruction following radiation and thermal exposure must indubitably have released into the blood stream protein split products, amongst these peptone, histamine, adenylic acid, and enzymes such as trypsin. The hemorrhage and shock following the injections of any of the substances mentioned are well known. I would like to know whether studies were made of the blood, either chemically or by injection into animals, to determine if such toxic products were actually released and whether or not it had been considered that these products might have attributed, in large measure, to the pathologic picture.

(Dr. Max M. Strumia, Bryn Mawr, Pa.) I would like to ask Dr. DeCoursey if any relationship was noted between the occurrence of intestinal lesions and the neutropenia, in view of the fact that in human patients suffering from malignant neutropenia or agranulocytosis, identical lesions are a common occurrence.

(Dr. Jacob Furth, New York, N.Y.) I would like to ask Dr. DeCoursey about the late effect of atomic bomb rays on the ovary, the organ in which neoplasms are most likely to occur after gamma irradiation. I also would suggest or question a correlation between the increased number of mast cells in tissues, as presented in the first paper, and the disturbance in blood clotting mechanism presented in the last.

(Dr. Austin M. Brues, Chicago, Ill.) Is it possible for Dr. Cronkite to give a breakdown of the 1300 and 1500 r. mentioned in his paper? Was that mostly at the time of blast from "gamma radiation," or was it partly from the beta- and gamma-emitting fission products dispersed at the time of the explosion and acting over a longer period of time?

(Dr. H. Edward MacMahon, Boston, Mass.) In view of what we have just seen and heard, it seems pertinent at this time to offer a word of warning about the indiscriminate use of radioactive substances in civilain life. It is one thing to use a radioactive substance for therapy in malignancy; it is quite another thing to use such a substance as a diagnostic aid. I have recently done an autopsy on a patient who received thorotrast,* which is radioactive thorium dioxide. This was given as a diagnostic aid. The autopsy showed not only very severe changes throughout the hematopoietic system similar to those that have just been described, but also a rapidly growing primary malignant endothelial cell sarcoma. This tumor arose in the liver in the immediate site of the greatest concentration of radioactive thorotrast in the body.

(Dr. Alfred Angrist, Jamaica, N.Y.) I would like to ask Dr. Wilder concerning the degree of change noted in these people with cataracts, and whether the patients who recovered showed changes in the lens, *i.e.*, whether the changes were sufficient to be recognized as subcapsular cataracts by the usual clinical means of detection.

(Dr. Liebow) In regard to the question about the mast cells, it was observed that these were numerous, not only in the lymph nodes, but also in the intestines of persons dying approximately 1 month after the bomb. They became a very prominent feature of the cellular content. This is of interest in relation to the observa-

* *Am. J. Path.*, 1947, 23, 585–611.

tion made by Dr. Jean Oliver of Brooklyn on mast cell tumors in dogs, from which a large quantity of heparin can be extracted. Among these animals there was no evidence of change in the coagulability of the blood, but we do not know the significance of this.

(Dr. DeCoursey) In regard to the question concerning the relationship between neutropenia and intestinal inflammation—yes, there usually was a direct relationship in that the patient had a history of previous leukopenia, usually long-continued. Three weeks after the Nagasaki explosion, Japanese hematologists reported zero white blood cells in about 26 per cent of a group of people who had been within 2,000 meters of the bomb.

In answer to Dr. Furth, the ovary shows surprisingly little effect. The primary follicles are numerous. The most constant finding is an absence of developing follicles. Hemorrhages are the only other feature.

A real difference between air and under-water detonation of an atomic bomb is that the radiations from an air explosion are instantaneous; prolonged ionizing radiations are emitted from the materials in the falling water resulting from underwater detonation.

(Dr. Wilder) I am sorry I cannot answer Dr. Angrist's question concerning the clinical manifestations of cataract and the ultimate results in patients who recovered, as we had no slit-lamp examinations on these patients, and the eyes examined at the Institute were all removed at autopsy.

(Dr. LeRoy) In answer to Dr. Rosenthal's question concerning the concentration of nonprotein nitrogen in the blood of the Japanese victims of the atomic bomb, some of the research groups studied this factor 3 to 4 weeks after the bombing, and found no significant variations. I am not aware of any estimations of the trypsin content of the blood. Such studies as were made of other secretions of the gastrointestinal tract were normal.

When I returned to Chicago after studying the atomic bomb material I discussed the matter of the hemorrhagic state with Dr. J. Garrott Allen whose demonstration of the presence of heparin-like material in the blood of experimentally irradiated animals was of great interest to me. I told him that our pathologic specimens contained unusual numbers of mast cells, an observation of considerable significance in view of the relationship between mast cells and heparin production. I was pleased to find that Dr. Cronkite was able to corroborate some of Allen's findings, since his observations are very significant. The demonstration that an anticoagulant substance occurs in irradiated subjects, and that this material can be neutralized by appropriate means, is very important from the clinical standpoint. There are many difficulties involved in the use of toluidin blue for this purpose, and investigations are being conducted in our own laboratory to find other, and more reliable means of accomplishing this.

(Dr. Tullis) In reply to Colonel Cornell's question, all types of food were exposed to ionizing radiations at Bikini. It was not fed to the animals because the food did not become radioactive.

(Dr. Cronkite) In regard to the question about histamine, peptone, and trypsin, we did not make such studies on our animals. These are contemplated in future experiments to be made on irradiated animals. The nonprotein nitrogen was not elevated.

In regard to the mast cells and the hyperplasia of them, I have not the slightest idea as to what the hyperplasia of the mast cells means.

In answer to Dr. Brues' question about the breakdown of the roentgen units, no one knows about that. Those recovered on the fourth day received 1300 r., and those on the fifth day, 1500 r. In respect to what Dr. LeRoy said, our confirmation of Allen's work is only partial. We did not directly isolate heparin. We titrated it indirectly with antiheparin agents.

THE PATHOLOGY OF SCHISTOSOMIASIS JAPONICA.* Mark M. Bracken and (by invitation) W. R. Bailey, Jr., Pittsburgh, Pa., and Henry M. Thomas, Jr., Baltimore, Md.

Abstract. During the early days of the occupation of the Philippine Islands by American troops in October and November, 1944, some of the troops were unavoidably exposed to water infested with *Schistosoma japonicum.* Although death rarely occurs in the acute stage of schistosomiasis japonica, 3 of these soldiers died and were examined post-mortem at overseas United States Army hospitals. In addition to the 3 cases studied at autopsy, specimens of acute lesions in the rectum, liver, skin, and brain were secured for biopsy from other patients. The older lesions of the disease, seen in 3 Filipinos who died in an American Army hospital following gunshot wounds, are included for comparison.

In the acute cases in this series ova have been found in the mesenteric lymph nodes, skin, brain, meninges, and adrenal medulla. They have been demonstrated in the late cases in retroperitoneal tissues, kidney, cerebellum, and medulla oblongata. In addition, lesions identical to those in which ova were demonstrated but in which eggs were not found were present in the myocardium.

The early lesions are usually miliary, appearing as yellowish white, caseous nodules or minute abscesses measuring from 0.5 to 10 mm. in diameter. In some of the lesions there is a necrotic zone around viable ova. This necrotic zone is surrounded by eosinophilic leukocytes and fewer neutrophilic leukocytes. As the lesion progresses, it presents central ova, either viable or degenerated with varying degrees of distortion and calcification, epithelioid cells, and fibroblastic proliferation in a richly vascular zone. Frequently the ova are partially or completely surrounded by multinucleated giant cells. The latter are usually of the foreign body type, but they may have the appearance of the Langhans' type. Later the eosinophilic leukocytes decrease in number and lymphocytes predominate.

The earliest lesions may coalesce to form large, irregular areas of necrosis in which are scattered the schistosoma ova. Fibroblastic and capillary proliferation begin early in the peripheral zone of the lesion, and in the older cases fibrosis predominates. The oldest lesions consist of shrunken, calcified ova surrounded by more or less dense fibrous tissue, with a moderate degree of lymphocytic cellular infiltration. In this pathologic picture the early lesions represent an unusual and characteristic reaction to the viable ova with necrosis and eosinophils, and the later lesions represent a foreign body reaction.

CORRELATION OF LABORATORY TESTS WITH THE PATHOLOGY OF SCHISTOSOMIASIS JAPONICA IN AMERICAN SOLDIERS. Stuart W. Lippincott and (by invitation), Lester D. Ellerbrook and Mark Rhees, Seattle, Wash.

Abstract. During the Leyte campaign in the Philippines a number of American soldiers were infected by the cercariae of *Schistosoma japonicum.* A group of 495 of these patients was studied with reference to tests of liver function, various types of stool examination, distribution and fate of antimony during and after treatment, clinical course of the disease, and the results of autopsies on 2 patients dying violent deaths during the period of investigation. A total of 17,295 examinations, using 8 technics, was made on 12,880 stools. The results showed that the direct smear in combination with one or more of the sedimentation methods detected more positive stools than if any one method was used exclusively for examination. In order to detect the maximum number of positive stools, the patient should be followed serially by stool examinations for a period of 10 consecutive days.

The lesions observed in the livers in the 2 autopsies consisted of either minute

* This article will appear in a subsequent issue of *The American Journal of Pathology.*

frank abscesses or fibrotic nodules. There was no indication that at this stage of the disease damage to the liver sufficient to impair its function had occurred. The initial studies of liver function in patients returning to this country from overseas showed the following incidence of abnormal results: globulin, 5 per cent; formol gel, 1 per cent; icterus index, 4 per cent; serum bilirubin, 6 per cent; urobilinogen, 0 per cent; intravenous hippuric acid, 5 per cent; galactose tolerance, 4 per cent; and bromsulfalein retention, 12 per cent. Repeated determinations of the bromsulfalein test and of the serum bilirubin concentration showed a definitely increased incidence of mild abnormalities toward the end of, and following, treatment with trivalent antimony compounds. In a small group of cases followed beyond 90 days, the incidence was markedly decreased.

When approximately 45 mg. of antimony were administered on alternate days, the plasma concentrations increased from about 8 μg. per liter to nearly 85 μg. after 3 weeks of treatment. After discontinuance of treatment the plasma concentrations decreased by 52 per cent in 12 days, and 80 per cent in 28 days. The concentration in blood cells was greater than that of plasma. The daily urinary antimony excretion increased from 2 mg. on the day of the first dose to roughly 10 mg. during the latter part of treatment. Forty days after discontinuance of treatment the daily excretion was still about 1 mg. The average 24-hour excretion in the feces varied from about 0.5 mg. per day during the first week to 2 mg. daily toward the end of treatment. Small amounts were still being excreted 100 days after treatment. The combined excretion in urine and feces toward the end of treatment was roughly 24 mg. in 2 days, or about 55 per cent of the amount administered in the preceding dose.

Tests of liver function performed before, during, and after treatment in these soldiers have shown that successive courses of antimony cannot be given with impunity. Furthermore, gross and histopathologic studies from 2 autopsies showed the hepatic lesions to be circumscribed with no evidence of fibroblastic proliferation, and that, even if antimony treatment failed to kill the worms, it would appear impossible to have enough seeding of the liver with eggs to destroy sufficient parenchyma to produce a true cirrhosis.

Discussion of Papers on Schistosomiasis Japonica

(Dr. H. M. Permar, Pittsburgh, Pa.) When Dr. Bracken sent us his first collection of autopsy sections, there were no data with them. However, we had our new Ash and Spitz, and so, comparing sections and text, we were able to alter our first opinion that we were dealing with tuberculosis in certain sections of the lungs and lymph nodes, and to arrive at the conclusion that we had lesions of schistosomiasis in all of the tissues. The sections are much more like tuberculosis than the areas selected for lantern slides. The important point, it seems to me, in Dr. Bracken's paper is the true nature of the early lesion. It is not easy to decide whether the ovum contains and gives off some toxic substance which causes necrosis, or whether it causes a polymorphonuclear exudate, chiefly eosinophilic, which then undergoes softening and breakdown. I rather incline to the latter view, and I believe Dr. Bracken leans to the former.

(Dr. Michael V. Mackenzie, Boston, Mass.) I should like to ask Dr. Bracken two questions. First, was the lesion which he showed that did not contain an ovum (even after serial sections) the only such lesion encountered in his case? Second, what significance does Dr. Bracken attach to this lesion (or lesions, if similar ones were also found)? Does he believe that it may be entirely a toxic reaction? Dr. Jaffé of Venezuela has reported that in experimental animals which have been infected only by male schistosomes, such lesions can occur. The implication, therefore, is that the presence of an ovum is not necessary for the pro-

duction of such lesions and that the adult schistosomes are truly pathogenic in their own right.

(Dr. Gustave J. Damin, St. Louis, Mo.) Dr. Lippincott has established the need for a large number of stool examinations in ruling out schistosomiasis in patients under treatment. When a concentration test is used, one should be selected which involves the sedimentation of a relatively large amount of feces. The schistosome eggs are classed among the heavier eggs and are not well concentrated by flotation technics.

(Dr. Bracken) In regard to Dr. Permar's question whether necrosis precedes or follows cellular exudate, we have seen in a very early lesion without cellular reaction a radial arrangement of filamentous material extending outward from the wall of a single ovum. This material could possibly be a lytic substance produced by the ovum.

Dr. Mackenzie, did you refer to the extensive lesion in the heart or to the very small one?

(Dr. Mackenzie) The very small lesion, the one which you stated did not reveal an ovum in serial sections.

(Dr. Bracken) It was just by chance that this lesion was present in a block removed from the heart. Perhaps the section not included in the block may have contained an ovum.

HISTOPATHOLOGY OF EXPERIMENTAL LEPTOSPIROSIS. Parker R. Beamer (by invitation), St. Louis, Mo., Hugh G. Grady, Philadelphia, Pa., and (by invitation) H. I. Firminger, Boston, Mass.

Abstract. Leptospirosis was produced in a relatively large series of apparently normal and healthy guinea-pigs weighing between 125 and 250 gm. Rectal temperatures and other clinical data were recorded prior to and during the course of the disease established by intraperitoneal inoculation of widely variable amounts of a culture of *Leptospira icterohaemorrhagiae,* isolated from a wild rat and maintained by animal passage and serial transfers in modified Schüffner's medium. At arbitrarily chosen, successively increasing intervals, animals were sacrificed, some being allowed to recover from the disease in order to study tissue from convalescing animals.

Multiple petechiae and larger foci of hemorrhage, particularly in the lungs and less commonly in the colon and adrenal glands, were observed within 48 hours. On the 4th day of the disease hemorrhagic foci were usually widespread, larger, and more numerous, occurring in the lungs, skin, and subcutaneous adipose tissue, serous membranes, gastrointestinal tract, kidneys, adrenals, epididymis, pancreas, retroperitoneal tissues, myocardium, and skeletal muscles. Cellular infiltration associated with hemorrhagic foci was minimal or absent. On the 5th and 6th days, as temperatures receded, generalized icterus developed. At this time hepatocellular disarray was prominent in the liver; necrosis of hepatic cells, individually or in small groups, and increases in mitotic figures were noted. At about this same period the kidneys were enlarged and pale, and degenerative changes with some necrosis of the proximal tubular epithelium were observed. There were small hemorrhages in a few glomeruli, and the distal and collecting tubules contained albumin and hyaline, cellular, and hemoglobin casts. Tissues from convalescent animals revealed little or no evidence of the disease.

Although hemorrhagic foci occurred in the myocardium, gastrointestinal tract, pancreas, brain, skeletal muscle, ovaries, testes, epididymides, and subcutaneous and retroperitoneal tissues, no leptospirae were demonstrated by silver impregnation, in association with these lesions. Organisms were present in largest numbers in the lungs within 48 hours. By the 4th to 5th days, leptospirae were found in

appreciable but decreasing numbers in the lungs, and the liver contained markedly higher numbers. From the 6th to the 19th days, large numbers were present in the renal tubules, whereas extremely few could be demonstrated in the other tissues.

Discussion

(Dr. Walter H. Sheldon, Atlanta, Ga.) I failed to catch whether you mentioned the animal used for your experiments. I wonder whether the natural course of the disease in your experimental animals showed relapses during the third week of the disease, which so commonly occur in human leptospirosis. Did you have any opportunity to study the changes of leptospirosis in the striated muscle, and were you able to demonstrate the organisms within these lesions?

(Dr. Ralph D. Lillie, Washington, D.C.) I failed to note in Dr. Beamer's presentation mention of the interstitial lymphocytic and other round cell infiltration in the kidney which occurs in human Weil's disease. We have seen it also in the experimental animals which we worked with some years ago.

(Dr. Beamer) In answer to Dr. Sheldon's question, the experimental animal used was the guinea-pig. In the relatively short observation period, no relapses were seen. We failed to find the lesion which you reported in striated muscle in human beings. I think, however, the course of the disease in our experimental animals was too acute to find degeneration and the subsequent changes which have been noted in human beings. We did find foci of hemorrhage in the muscles, and in the animals which recovered from the disease we found no additional changes during the relatively short period of observation. In a few instances in which the attempt was made, leptospirae were not found in the muscular lesions.

In answer to Dr. Lillie's question concerning the infiltration of cells in the kidney, that was noted in many instances, but usually not to a significant degree. We found, in general, that interstitial infiltration in the kidney was only of slight degree in these animals, and infiltration of similar extent was found in an appreciable number of control animals of comparable ages.

THE RECIPROCAL RELATIONSHIP OF SURFACE TEMPERATURE AND TIME IN THE PRODUCTION OF HYPERTHERMIC CUTANEOUS INJURY. A. R. Moritz and (by invitation) F. C. Henriques, Jr., Boston, Mass.

Abstract. The threshold for the occurrence of irreversible epidermal injury at surface temperatures varying between 44 and 100° C. was observed in porcine and human skin. Surface temperature was maintained at a constant predetermined level by contact with a rapidly flowing stream of hot liquid. The time required to produce irreversible injury bore an inverse relationship to temperature. The effect of circulation of blood through the dermal capillaries on the susceptibility of the skin to thermal injury was investigated. The causation of rapidly fatal circulatory failure incident to generalized cutaneous exposure to excessive heat was determined.

Discussion

(Dr. Raymond H. Rigdon, Little Rock, Ark.) What was the age of the animals you used, and was the hair removed before you made these observations?

(Dr. Herbert Lund, Cleveland, Ohio) Was there a difference in the injury of the deeper tissues at different temperatures? Cooking a roast beef at low temperature for a long time produces changes throughout most of the meat, and we know that the same effect is not produced by a flash of heat of high intensity. In these injuries of the skin I would be interested to know if a long duration of low temperature heat produces a deeper effect.

(Dr. Moritz) The pigs we used weighed between 10 and 15 kg. and it was found that the lateral thoracic and lateral abdominal areas were uniformly responsive.

There are other sites that do not respond uniformly, and which should be avoided. The pigs were clipped, although this is not necessary.

Dr. Lund's question has to do with the establishment of gradients through the skin, and time did not permit me to discuss the measurement of skin gradients. The higher the surface temperature, the steeper the trans-cutaneous gradient becomes and the shorter the time required to destroy the epidermis. As surface temperature is increased, the damage to the deeper tissues becomes relatively less severe than that to the superficial cells. You can char the surface of the skin with no change in the dermis, if you are using a high enough temperature. The long exposure time required to kill the epidermal cells at the lower temperatures predisposed to a greater depth of injury.

QUANTITATIVE HYPOTHERMAL PRODUCTION OF CLOSED CEREBRAL INJURY. G. M. Hass and (by invitation) C. B. Taylor and J. E. Maloney, Chicago, Ill.

Abstract. A method for producing quantitative controlled destruction of local areas of the cerebral cortex of rabbits by the use of a freezing device applied to the external surface of the intact skull is described. Cylindrical lesions of the cortex varying from 2 to 25 mm. in diameter and 1 to 7 mm. in depth, even extending through the white matter into the lateral ventricles, may be produced. The breadth and depth of the lesions depend upon the dimensions of the tip of the freezing device and the duration of contact of the tip of the device with the skull. The lesions resemble strictly localized infarcts or contusions of the brain. In single acute experiments, survival of the animal depends upon the volume and depth of the lesions. By production of successive sublethal lesions, over 30 per cent of the brain can be progressively destroyed without opening the skull, or introducing any factor such as widespread hemorrhage or concussion. An experimental approach to therapy of closed intracerebral hemorrhage with necrosis of the brain by a quantitative method becomes possible.

Discussion

(Dr. A. R. Moritz, Boston, Mass.) I would like to inquire as to the mechanism of the injury. Is this tissue frozen? Does it die because of intracellular congelation, because of interference with circulation, or is there some other injurious mechanism?

(Dr. Jacob Werne, Jamaica, N.Y.) I wonder whether the exact location of the traumatic lesion was considered in evaluating these experiments. In medicolegal practice it is well known that the site, no less than the extent of the lesion, frequently has a bearing on the outcome of a given case.

(Dr. Alfred Angrist, Jamaica, N.Y.) Is there any evidence, in the clinical sense, of increased intracranial pressure in these animals? In other words, is the degree of edema sufficient to alter the dynamics of the cerebrospinal fluid, and were any changes found in the pons in these animals? With human head injuries it is well known that about one-third of the cases will show pontine hemorrhages.

(Dr. Taylor) The tissue has been destroyed primarily by the low temperature. The cooling plate has a temperature of $-50°C$.

In reply to Dr. Werne, there is very little hemorrhage in the damaged tissue, and very little reaction around the lesions. All damage was confined to the cerebrum in this group and there was no relation between the site of injury and death. There was no difference whether the lesion was on the left or the right side. If lesions extended into the midbrain, animals usually did not survive. We confined our studies to the cerebral cortex for that reason.

Dr. Angrist asked about increased intracranial pressure. We have not been able to measure the cerebrospinal fluid pressure in rabbits. Animals did exhibit symptoms of increased intracranial pressure. We have found no changes in the pons.

We have had a few rabbits with a very small amount of subarachnoid hemorrhage, just over the fourth ventricle.

THE DISTRIBUTION OF BRAIN STEM LESIONS IN POLIOMYELITIS. John C. McCarter and (by invitation) M. Barnhart, R. Rhines, and H. W. Magoun, Evanston, Ill.

Abstract. This report is concerned with the detailed anatomic distribution of the lesions of acute poliomyelitis in human brain stems, and the correlation of clinical symptoms. The material of the inquiry was 7 brains and cords obtained at autopsy from patients dying of acute poliomyelitis at Evanston Hospital in the epidemics of 1943–44–45. Sections from several areas of cerebral cortex, cerebellum, and spinal cord, together with serial sections of the entire brain stem, have been examined, and the distribution of lesions plotted in each case. All 7 cases (6 of bulbar type and 1 of spinal type, clinically) had severe damage to the reticular formation of the brain stem. This constant pattern of involvement is regarded as of prime importance in the causation of symptoms of respiratory impairment, vasomotor collapse, and muscle spasticity exhibited by patients dying of acute poliomyelitis.

THE CHANGES IN THE MOTOR CORTEX IN ACUTE POLIOMYELITIS. Kornel L. Terplan, Buffalo, N.Y.

Abstract. In 49 of 56 cases of acute poliomyelitis, distinct recent changes were found in the motor area, particularly in the third to sixth layer, occasionally extending into the adjacent subcortical white matter. These changes were readily seen with the naked eye in preparations stained with thionin or toluidin blue, and, in particular, small nodular infiltrations and micro-abscess-like lesions about dead or necrobiotic nerve cells. Histologic analysis revealed the same type and frequently the same intensity of cellular damage and inflammatory reaction as in the anterior horns of the spinal cord and in the brain stem. The degenerative changes involved the pyramidal cells in the cortex, including the Betz cells, leading occasionally to complete disappearance of giant pyramidal cells and their replacement by small triangular glial nodules. There was an excessive activation of microglial cells which, together with disintegrating leukocytes, formed the usually ill defined abscess-like lesions. In some cases there was band-like vertical infiltration extending upward into the second or, rarely, into the first layer. In other cases there was more diffuse proliferation of microglial cells and extensive neuronophagia. Perivascular cuffing in the motor cortex and the adjacent subcortical white matter was a prominent feature. In a few instances there were small areas of recent softening with masses of fat granular cells about veins in the fifth and sixth layer of the cortex, accompanied by segmental disappearance of all nerve cells in this area. All changes were most pronounced in the posterior portion of the precentral gyrus. The various reactive inflammatory changes in the supporting mesodermal and ectodermal tissue were similar to the acute inflammatory response in the anterior horn of the spinal cord, medulla, dentate nucleus of the cerebellum, tegmental portion of the pons, and in the subthalamic and thalamic area. Various representative areas from other parts of the pallium were examined, including the frontal, occipital and parietal lobes, Ammon's horn and hippocampal gyrus, and the island of Reil, and no similar cortical changes were seen. In comparison to the distinct damage revealed in the motor cortex, the remainder of the pallium could be considered as negative.

This selectivity of the poliomyelitic virus for the neurons of the motor cortex appears just as characteristic in our experience as the marked damage of the nerve cells in the anterior motor horns. As far as the pallium is concerned, it is diagnostic of acute poliomyelitis. Selectivity for the motor cortex has been observed following

intracerebral inoculation of the monkey with the human virus by Hurst, Luhan, and Peers. Findings apparently identical with ours had been observed in human poliomyelitis by Andre-Thomas and Lhermitte, by Spielmeyer, and especially by Koernyey.

In the 7 remaining cases there were only minimal findings in the motor cortex, including neuronophagia about a few small pyramidal cells and very slight pericapillary round cell infiltration. In all 56 cases acute poliomyelitic damage was present at various levels of the spinal cord, in the medulla and pons, and in those areas of the peduncles, hypothalamus, globus pallidus, and thalamus which usually are found involved in acute poliomyelitis.

The selective involvement of the motor cortex was observed not only in the majority of the cases from the epidemic in Buffalo in 1944, but also in isolated cases occurring in the years 1937, 1939, 1941, 1943, 1945, and 1946.

Discussion of Papers on Poliomyelitis

(Dr. Russell L. Holman, New Orleans, La.) I would like to ask Dr. McCarter the age of the patients and the duration of the disease; also whether there was any clinical correlation with the distribution of the lesions.

(Dr. McCarter) First, as to the age of the patients: the youngest in this series was 2½ years, the oldest was 46, with a large proportion of the entire 18 cases ranging below 20 years. As to the time interval from the onset of the disease to the time of death, that varied between approximately 36 hours in the shortest case, to 23 days in the longest one. I did not quite understand your second question.

(Dr. Holman) Was there any correlation between the duration of illness, clinical symptoms, and the type of death and the location and extent of the lesions?

(Dr. McCarter) I think there was, very definitely. We were impressed with the large proportion of these cases which showed circulatory collapse, which showed respiratory collapse, pulmonary edema, and lack of tonus of the gastrointestinal tract. In these fatal cases these symptoms were much more prominent than in the ones who survived, my clinical colleagues tell me, but many of those who survived showed varying degrees of these symptoms.

POST-MEASLES ENCEPHALOPATHY. Karl T. Neubuerger, Denver, Colo.

Abstract. The pathologic picture of acute post-measles encephalitis has been established, but lesions of the brain in more chronic cases have been reported in only a few instances. The findings in 2 patients who survived the acute stage are presented. The first case was that of a 60-year-old man who had measles and, 10 days later, lapsed into a semistuporous state with generalized spasticity and progressive mental deterioration. He died 10 weeks after onset of the cerebral symptoms. The second case was that of a 7-year-old boy who became semicomatose, with fever, rigidity, and shaking, 10 days after having been taken ill with measles. He died 6 weeks later. The immediate cause of death in both patients was bronchopneumonia. The nervous lesions were similar. The essential site was the cerebral white matter where patchy, sometimes coalescing foci of perivenous demyelination were found. The axis cylinders were damaged to a less extent than the myelinated fibers. The foci were not well defined; some were coarsely vacuolated, particularly in the older patient. Proliferated microglial cells were laden with lipoid disintegration products of the destroyed nerve fibers. In some fields, areas of softening with densely packed gitter cells were observed. Large astrocytes were present within the foci and in their immediate vicinity. In what appeared to be older lesions they had superseded the microglial elements. Glial fibrils forming a coarse network were numerous in the older case, but sparse in the younger one. Some of the intrafocal veins exhibited stasis or recent thrombosis. In addition to the changes described, the brain of the boy showed a few scattered foci

with plasmatic gliosis in the extracortical gray matter, with very little damage to the nerve cells. There was also some marginal gliosis in pons, medulla, and upper cord.

As to the pathogenesis of this condition, the following statement can be made. In a limited number of patients with measles the causative agent, possibly a virus, reaches the brain probably by way of the blood stream, and displays its activity mainly in the white matter which is less well vascularized than the gray. It causes disturbances in the terminal circulation, particularly on the venous side. Apparently it diffuses through limited perivenous zones only. It is possible that allergy plays some part since experimentally produced allergic lesions have similar histologic features.

The relation of post-measles encephalitis to multiple sclerosis is as yet an unsolved problem. The findings in the cases of encephalopathy under discussion do not favor the assumption of the identity of both diseases, despite many similarities. Some of the features present in post-measles encephalopathy but not in multiple sclerosis are lack of predilection for the periventricular region; scarcity of cortical foci; strictly perivenous location; lack of sharp demarcation of the foci; more damage to the axis cylinders; occurrence of fibrillary gliosis only in the later stages; lack of "advancing cell wall," inflammatory features, and mesenchymal proliferation as seen in recent foci of multiple sclerosis; absence of appreciable variation in the age of the foci; and definite difference in the clinical picture.

Discussion

(Dr. John C. McCarter, Evanston, Ill.) What symptoms in the clinical course of these patients were ascribed to post-measles encephalitis? They died, of course, of bronchopneumonia, which I assume was not related to the disease.

(Dr. Neubuerger) The bronchopneumonia was not related to the disease. The main symptom was the very marked spasticity in both cases.

HISTOLOGY OF CORONARY ARTERIES IN NEWBORN INFANTS. R. J. Fangman (by invitation) and C. A. Hellwig, Wichita, Kans.

Abstract. In a recent paper, William Dock suggested that the preponderance of males dying of coronary disease might be explained by inherited variations in the thickness of the coronaries. He found the intima of coronary arteries to be much thicker in newborn males than in females. Dock concluded that because of this anatomic peculiarity it has already been determined at birth whether coronary occlusion may occur.

We examined during the past year the coronary arteries of 30 newborn infants. Sections were made from the three main vessels of 15 males and 15 females. The frozen and paraffin sections were stained by different methods in order to study not only the cellular and fibrous elements, but also accumulations of lipoid and hyaline material. In 12 cases, 8 males and 4 females, we found thickening of the intimal layer most often in the anterior descending coronary artery, but never involving the total circumference of the vessel. The cushion-like elevations varied from small buds to more than one-half of the circumference of the vessel. Verhoeff's stain for elastic fibers revealed small breaks in the internal elastic lamella, or it was composed of frayed elastic fibers. In some cases it was split into two layers and under several elevations the elastic lamella was absent. No less revealing were the sections stained with sudan. We found fine deposits of lipoid along the elastic fibers and in the stroma of the cushions and sometimes also in large histiocytes.

While our findings are identical with those of Dock, concerning the presence of cushion-like thickening of the coronary arteries in newborn infants, our interpretation differs from his view that these are inherited anatomic peculiarities. The

presence of lipoid deposits and the destruction of elastic tissue throw serious doubts on the explanation offered by Dock. We believe that these are pathologic processes, *i.e.*, the earliest stages of intimal arteriosclerosis. Our standpoint is supported by the facts that the thickenings are restricted to certain areas of the intimal circumference, that they are most common in the anterior descending branch, and that they are more often found in male infants, characteristics which apply to coronary sclerosis of the adult as well.

The degeneration of the elastic fibers under the cushions is caused most likely by the deposits of lipoid. According to Hueper, deposits of macromolecular colloids in tissue interfere with the normal exchange of nutritive substances between tissue and plasma. The proliferation of fibroblasts is then a reparative process following injury of the tissue by accumulation of lipoid. We regard the precipitation of lipoid as the primary event in the formation of these cushions.

Discussion

(Dr. Theodore J. Curphey, Hempstead, N.Y.) I should like to ask whether these changes were more noticeable in the proximal third of the anterior descending branch than in the rest of its course. In many cases of sudden death secondary to coronary occlusion, we have seen the occluding arteriosclerotic process confined to the first 2 or 3 cm. of the anterior descending branch.

(Dr. Hellwig) We found most of the cushion-like elevations in exactly those locations which Dr. Curphey pointed out. Only in the epicardial portions of the coronaries, never in the muscular branches, do these lesions occur.

(Dr. Russell L. Holman, New Orleans, La.) I would like to ask if you found these changes in other vessels, particularly around the aortic valves and around the mouths of the coronary vessels; also whether there was any correlation between the deposits in the coronary vessels and elsewhere in the large vessels.

(Dr. Hellwig) We did not search systematically for lipoid deposits in other blood vessels, and I am unable to answer Dr. Holman's questions. However, in former studies on the atheromatosis of the mitral valve, I noticed distinct lipoid spots in the mitral valve already in the second month of life.

(Dr. S. Milton Rabson, Fort Wayne, Ind.) What was the relationship between the disease from which the newborn infants died and the degree of severity of the process described?

(Dr. Hellwig) In our positive cases the cause of death varied. We were not able to establish any relationship between cause of death and presence or degree of lipoid deposits in the coronaries.

(Dr. Howard T. Karsner, Cleveland, Ohio) Do you attach any significance to the difference in the incidence in the two sexes which you have observed?

(Dr. Hellwig) I believe that the sex difference is significant. The view of Dock that males have a slower pulse and higher stroke volume seems supported by the fact that in male newborns the weight of the heart is slightly higher than in females. The predilection of these lipoid deposits for the anterior descending coronary of males could well be explained by a more vigorous vibration of the vessel wall during systole disturbing the colloidal stability of the lipoid dispersion in the intima.

THE EFFECT OF PATENT DUCTUS ARTERIOSUS ON THE DEVELOPMENT OF PULMONARY VASCULAR LESIONS.* Thomas D. Kinney and (by invitation) Kenneth J. Welch, Boston, Mass.

Abstract. The effect on the pulmonary vascular bed of an abnormal communication between the systemic and pulmonic circulation was studied in a group of patients having uncomplicated patent ductus arteriosus. No pathologic lesions in the small branches of the pulmonary artery were observed in 27 patients with

* This article will appear in a subsequent issue of *The American Journal of Pathology.*

uncomplicated patent ductus arteriosus. This evidence suggests that the belief in the common occurrence of pulmonary vascular disease resulting from a patent ductus arteriosus is erroneous.

MYOCARDITIS IN EARLY LIFE. Reginald K. House (by invitation), Hanover, N.H.

Abstract. The gross and microscopic findings of 5 cases of myocarditis of obscure origin in infants are described. The various etiologic factors are mentioned, with emphasis on possible hypersensitivity or virus infection.

Discussion

(Dr. I. Davidsohn, Chicago, Ill.) I did an autopsy just a few weeks ago on a baby, age 26 hours, in whom I found an acute diffuse myocarditis. The baby was born after an uneventful pregnancy, and showed, since birth, clinical manifestations that were interpreted as meningeal hemorrhage. No other lesions were found at autopsy. The endocardium was normal; the myocardial lesions were visible to the naked eye through the epicardium as distinct yellowish gray areas, mainly on the anterior surface of the left ventricle.

CONGENITAL HEART DISEASE WITH NECROTIZING ARTERITIS (PERIARTERITIS NODOSA) LIMITED TO THE PULMONARY ARTERIES: REPORT OF CASE WITH NECROPSY. Jacob W. Old (by invitation) and William O. Russell, Santa Barbara, Calif.

Abstract. An 11-year-old California boy of Mexican parentage, known to have congenital heart disease from birth, died after an illness of 30 days. He had enjoyed good health until his terminal illness, except for moderate limitation of physical activity imposed by his disease. His principal symptoms were attacks of headache, malaise, and on two occasions a slight elevation of temperature. Terminally, there was excessive perspiration and a dry cough, and a tentative diagnosis of pneumonia was made. Treatment with oral penicillin was unsuccessful because of vomiting. Bright red blood, presumed to be of pulmonary origin, found on his clothing led to hospitalization in a tuberculosis sanatorium. Physical examination revealed a lethargic, cyanotic boy with rapid pulse and respirations and a diastolic apical heart murmur. A roentgenogram of the chest showed soft infiltrations, varying from 1 to 5 mm. in diameter, in all pulmonary lobes. The family and personal histories disclosed no known sensitizations or symptoms of allergic states, and there was no known therapy with sulfonamide drugs. A polymorphonuclear leukocytosis of 20,000 was present before death.

Necropsy revealed a large defect in the membranous part of the interventricular septum with marked hypertrophy and dilatation of the right ventricle, and moderate chronic mitral endocarditis. In the lungs the medium-sized arteries contained recent and partially organized thrombi with a moderately broad zone of induration surrounding the involved vessels. Microscopic studies of the lungs showed acute necrotizing arteritis of the arterioles and of the medium and small arteries with hyalinization and fibrinoid change in the wall accompanied by infiltrations of polymorphonuclear leukocytes. The most acute lesions were frequently limited to focal necrotic changes in the arterial wall. Generally, however, there was complete necrosis of the arterial walls in which the lumina were filled with thrombi, occasionally showing organization. The arterial walls and adventitial tissues were infiltrated with polymorphonuclear leukocytes and macrophages but no eosinophils. Some of the arteries showed organized thrombi with recanalization. Arteries and arterioles in sections taken from all other viscera except the brain, which was not examined, were normal. The necrotizing arteritis was regarded as characteristic of the lesions observed in periarteritis nodosa occurring in man and experimentally produced in sensitized animals.

The localization of the arteritis to the pulmonary arteries was explained on the basis of a greater exposure of these arteries to an unidentified agent causing the

arterial disease of periarteritis nodosa. The defect in the interventricular septum caused a proportionately greater circulation through the right side of the heart and pulmonary arteries than through the systemic arterial system. This case suggests that the blood concentration of the unidentified agent producing necrotizing arterial disease is an important factor in the genesis of the lesion and that a critical concentration must be reached to produce the disease.

Discussion

(Dr. Howard T. Karsner, Cleveland, Ohio) I would like to put this in the form of a question, although it might seem that I am delivering a lecture on the subject of arteritis. It seems to me that if we call all forms of acute arteritis "periarteritis nodosa," we are probably confusing ourselves, or at least not permitting ourselves to be specific in the identification of arterial lesions. In my opinion this case does not fulfill the usual criteria that we apply to periarteritis nodosa, which is a systemic disease. To be sure, its manifestations may be more pronounced in one situation than in another, but even temporal arteritis is now known to be something more than a local disease. I think we should pay some attention, although this is purely a personal opinion, to the infiltration of eosinophils in the exudate. In this particular instance there was no eosinophilia of the circulating blood and, as far as I can learn, there was no eosinophilia in the exudate. Whether we argue for or against hypersensitivity or allergy in connection with periarteritis nodosa, nevertheless it is a widespread disease. We realize, however, that in rheumatic vascular disease there may be a much greater degree of localization. We see it in the myocardium in outspoken, florid rheumatic fever, and in the myocardium lesions are found which are microscopically identical with those exhibited in this case. They occasionally occur in the brain, and of course we all have in mind the discussion concerning the manifestations of rheumatic fever in the lung. With this preliminary lecture, I ask why this should not be classified simply as rheumatic vascular disease in the lung.

(Dr. Old) In our study of the literature of this subject we were first impressed with the fact that there is great overlapping of the lesions of rheumatic disease, periarteritis nodosa, and other forms of arteritis. It was for this reason that the term necrotizing arteritis was used and periarteritis placed in parentheses in the title. This seemed the most logical way to regard these lesions until more definite and specific diagnostic criteria are available for the anatomic diagnosis of rheumatic arteritis. We do not feel that the question can be answered categorically. Against a rheumatic type of arteritis is the fact that no evidence of active rheumatic disease was found in the heart or other organs. We should like to emphasize that this case was presented merely as one in which there was evidence of previous rheumatic infection and in which necrotizing arteritis occurred in only one organ. The localization of the lesions in arteries previously altered as a result of the patent interventricular septum, changes that Dr. Kinney has so well described this afternoon, is an interesting observation that we hoped would stimulate further comment for our better understanding of the unknown factors giving rise to them.

CLINICOPATHOLOGIC AND EXPERIMENTAL OBSERVATIONS ON THE PATHOGENESIS OF RUPTURE OF THE HEART DUE TO MYOCARDIAL DAMAGE. W. C. Thomas, Winston-Salem, N.C.

Abstract. In addition to the anatomic considerations of size, site, age, and histologic type of infarction, there are numerous physiologic factors concerned in rupture of the heart. Some of these are intracardiac pressure, tensile strength of the wound produced by the injured muscle, and the contractility of the adjacent undamaged musculature. An analysis of the relationship of these various factors and the clinicopathologic findings was made.

Attempts were made to determine the influence of various forms of exertion in

the production of heart rupture. Myocardial damage was produced in rats by searing the anterior surface of the left ventricle. An experiment was carried out in which one group of animals was exercised forcibly by swimming, while another group was allowed to exercise voluntarily. No difference was noted in the incidence of heart rupture in the two groups. Another series of animals was studied to determine the influence of varying obstruction to the inspiratory and expiratory phases of respiration. No ruptures of the hearts occurred even though the period of maximum occurrence served as the time of testing. Finally, the amounts of intraventricular pressures necessary to cause ruptures of the hearts were measured on the tenth day following muscle damage. The pressures varied between 150 mm. and over 400 mm. of Hg. The average pressure was found to be 225 mm. of Hg.

Discussion

(Dr. Howard C. Hopps, Oklahoma City, Okla.) No mention was made of the extent or the type of mural thrombus that might follow infarction and thus reinforce the weakened region. I wonder if that might not be an anatomic factor in protecting the heart from rupture.

(Dr. Jesse E. Edwards, Rochester, Minn.) I would like to ask Dr. Thomas whether he made any correlation between the amount of necrosis and the incidence of rupture. The point I have in mind is whether the necrosis extended to the endocardium. I think every one of us here has seen that in most instances of rupture of the heart of human beings the myocardial necrosis is extensive and frequently involves the muscle down to the endocardium, in contrast to the usual myocardial infarction in which there is no infarct in the subendocardial muscle.

(Dr. Thomas) In reply to Dr. Hopps' remarks, I wish to say that I encountered no mural thrombi in the hearts of these animals.

In connection with Dr. Edwards' question I found that there was generally more extensive necrosis and less connective tissue response in the hearts which ruptured. In these cases the necrosis extended more deeply into the subendocardial tissues than in the hearts which did not rupture.

OBSERVATIONS ON THE LUNGS OF THE NEWBORN. J. Edgar Morison (by invitation), Belfast, Ireland.

Abstract. The morphologic state of the lungs at birth may influence the postnatal picture, but, while considerable variation in the degree of expansion of the air spaces *in utero* has been found, the normal range of variation is not known. In the production after birth of the irregular pattern of initial atelectasis, the imporance of variations in fluid absorption from the peripheral parts of the respiratory system and of irregular air entry is emphasized. Secondary edema may further modify the picture. The distribution of edema fluid in the interstitial tissue of the partitions between the air spaces, as studied in the lungs of premature infants, suggests that normally the air spaces are separated from the mesenchyme by a continuous membrane. Cellular reactions in the lung in autopsies from before and after the era of specific chemotherapy have been studied with special reference to mononuclear reactions. Reactions actually in the interstitial tissue are slightly more marked in some of the recent cases, but the significance of this is still uncertain.

THE PATHOGENESIS OF CONGENITAL POLYCYSTIC LUNG AND ITS CORRELATION WITH POLYCYSTIC DISEASE OF OTHER EPITHELIAL ORGANS. RECONSTRUCTION OF CYSTIC ELEMENTS IN TWO CASES.* Robert F. Norris and (by invitation) Ralph M. Tyson, Philadelphia, Pa.

Abstract. The polycystic lesions in the lungs of 2 infants are described and reconstructions of the cystic elements are illustrated. The fundamental lesion ap-

* This article will appear in a subsequent issue of *The American Journal of Pathology.*

pears to be focal segmentation preceded or followed by focal dilatation of the small bronchi and bronchioles. If the disease becomes arrested in the stage of focal dilatation without segmentation, bronchiectasis results which may persist for the duration of life. When the bronchi are broken up into isolated segments, some of these segments persist as gradually enlarging cysts. The well known lesions of polycystic disease are thus established. The lesions and sequence of anatomic changes in the polycystic lung are similar to those previously described in the polycystic kidney, liver, and pancreas. In general the lesions of polycystic epithelial organs primarily affect the nonfunctioning system of ducts. By analogy with the process of physiologic resorption in normally provisional organs or parts of organs, it is concluded that polycystic disease is a pathologic manifestation of normal fetal resorption and degeneration. The polycystic organ is therefore partially provisional. The fundamental defect which initiates the developmental anomalies is unknown and the etiologic importance of anomalies of circulation in polycystic organs has not been determined.

SPONTANEOUS AND INDUCED GLOMERULONEPHRITIS IN AN INBRED STOCK OF MICE. Arthur Kirschbaum (by invitation) and E. T. Bell, Minneapolis, Minn.

Abstract. Mice of the NH strain develop glomerulonephritis spontaneously. The structural changes in the glomeruli closely resemble those found in human glomerulonephritis. In the acute stage, which may be seen in some kidneys, the capillaries are filled with endothelial cells. The majority of the affected glomeruli are in the chronic stage and show endothelial proliferation with thickening of the intercapillary septa. Associated with the renal lesions are albuminuria, edema, low plasma protein levels, nitrogen retention, anemia, and occasionally hypercholesterolemia.

This stock of mice developed glomerular lesions following the administration of urethane (1 mg. per gm. of body weight in 10 per cent aqueous solution administered intraperitoneally once a week for 4 to 6 months). The glomerular lesions were not identical with those of human glomerulonephritis, but resembled those of lipoid nephrosis in that there was thickening of the capillary basement membrane and only a moderate endothelial proliferation. There were numerous thromboses in the glomerular capillaries. The urethane-injected animals with advanced glomerular lesions were edematous, exhibited albuminuria, low levels of plasma proteins, and renal insufficiency (elevation in blood urea nitrogen).

THE JUXTAGLOMERULAR APPARATUS IN EXPERIMENTAL HYPOTENSION. F. W. Dunihue (by invitation), Burlington, Vt.

Abstract. The function of the juxtaglomerular apparatus (JGA) is an open question; yet there is considerable indirect evidence in support of Goormaghtigh's hypothesis that the cells of the JGA secrete renin. The principal basis of this hypothesis is that the increased renin activity found in the early stages of experimental renal hypertension is accompanied by hypertrophy of the JGA, and, in most animals, by an increase in fuchsinophilic granular cells.[1] Since an increased renin activity has been reported in a variety of hypotensive states, such as hemorrhagic shock and adrenal insufficiency, it is to be expected on the basis of the above hypothesis that the JGA would be hypertrophied and contain increased granular cells in these conditions. The experiments here reported are in general agreement with this expectation.

Hemorrhagic shock was induced in 20 rabbits using Wolcott's method. Fatal shock under these conditions produced changes in the JGA which varied with the duration of the shock. In rabbits dying within 2 to 5 hours, there was an increase in the granular cells of the apparatus; the granules were characteristically finer, less numerous, and stained differently than those seen subsequent to procedures which produce experimental renal hypertension. In fatal shock of shorter duration

the increase in granular cells was not so clear, but the number of such cells in all animals was slightly above or at the upper normal limits.

Bilateral adrenalectomy was performed in two stages in rabbits, cats, dogs, and monkeys. The JGA in all these animals was hypertrophied, hyperplastic, and contained increased fuchsinophilic granular cells, which morphologically and tinctorially were similar to those seen in experimental renal hypertension.

The stimulus provoking these reactions in the JGA cannot be solely hypotension, since hypoxia also was present. However, the fact that these changes were present in adrenalectomized monkeys prior to an actual drop in the blood pressure and in rabbits which show no signs of deficiency suggests hypoxia or some other factor as the effective stimulus.

It is not clear at the present time whether these juxtaglomerular changes can be related to VEM which is reported to be of renal cortical origin by Shorr and his colleagues.

Discussion

(Dr. N. Goormaghtigh, Ghent, Belgium) I think it is only natural that I should comment on the work of Dr. Dunihue, and that I should congratulate him for the work he has done. In research there is always a human side, and it is very gratifying for a research worker to find that somebody has confirmed and extended the observations which he has made originally. I should like to say that those who are interested in the problem can very well follow the technic used by Dr. Dunihue. I think his technic is better than mine, and it is advisable that his directions should be followed. I should like also to emphasize the importance of the factor which he has underlined, namely, renal anoxia as a cause of the increase of the arteriolar secretory cells.

THE PERIODIC ACID ROUTINE APPLIED TO THE KIDNEY.* J. F. A. McManus (by invitation), Birmingham, Ala.

Abstract. Selective staining of the basement membrane of the renal tubules and glomerulus of the normal kidney is produced by treating sections with periodic acid and then with Schiff's reagent for aldehydes. In abnormal kidneys there is coloring not only of the basement membrane, but also of the hyaline droplets in the tubular epithelium, the hyalin of arteriolosclerosis, and the granular cells of the renal arterioles. This coloring of basement membrane and other structures in the kidneys may depend upon the production of aldehyde by periodic acid from the carbohydrate moiety of mucoprotein, since periodic acid has the ability to produce aldehyde from carbohydrate. However, histochemical interpretations should be deferred, because serine, threonine, and hydroxylysine also form aldehyde when acted upon by periodic acid and because many compounds of carbohydrate joined to proteins or to lipids are present in tissues.

Discussion

(Dr. N. Goormaghtigh, Ghent, Belgium) I should like to ask if the use of periodic acid gives any indication of the chemical structure of the granules found in the cells of the juxtaglomerular apparatus.

(Dr. McManus) Two groups of materials are known to color with Schiff's reagent after periodic acid: (1) Carbohydrates, either in combination with protein or polymerized, and (2) three amino acids that Nicolet and Shinn have found. However, the chemists think that these three amino acids will have to be in a terminal position in order to be attacked by periodic acid, so it is quite within the realm of probability that a carbohydrate or a carbohydrate compound is being demonstrated in the granular cells of the renal arterioles.

Since preparing this paper we have been able to find the renal arteriolar granular

* This article will appear in a subsequent issue of *The American Journal of Pathology*.

cells in a wide variety of conditions which we are not yet able to explain fully. Apparently in certain conditions autolysis is not so rapid as it is in others, as regards the granular cells, so with this method the survey of many kidneys may be worth while.

THE PANCREAS IN UREMIA. Archie H. Baggenstoss, Rochester, Minn.

Abstract. Histologic examination of the pancreas at necropsy in cases of uremia frequently revealed varying degrees of dilatation of the acini, flattening of the lining epithelial cells, and inspissation of intra-acinar secretion. Investigation showed that the lesion was present throughout the pancreas and was not associated with obstruction of the ducts. Cells of the involved acini had lost their zymogen granules and stained uniformly pink with hematoxylin and eosin. The material in the acini appeared amorphous, stringy, or laminated and also stained pink with this stain. It reacted like protein to a variety of stains. It occasionally contained cellular débris. When a half or more of the acini were involved, the lesion was classified as severe; when many, but less than half, of the acini were involved, the lesion was considered as moderately severe; and when only a few acini in each lobule were involved, the lesion was classified as mild.

The lesion was found in 33 (39 per cent) of 85 cases in which chronic glomerulonephritis terminated in uremia. The lesion was classified as mild in 19 (58 per cent) of the cases, as moderately severe in 9 (27 per cent) of the cases, and as severe in 5 (15 per cent) of the cases. The lesion was found in 36 (42 per cent) of 85 cases in which hypertension terminated in uremia. The lesion was classified as mild in 22 (61 per cent) of the cases, as moderately severe in 12 (33 per cent) of the cases, and as severe in 2 (6 per cent) of the cases. The lesion was found in 52 of 100 cases in which uremia resulted from miscellaneous causes, such as urinary obstruction, pyelonephritis, and extrarenal factors. The lesion was classified as mild in 28 (54 per cent) of the cases, as moderately severe in 16 (31 per cent) of the cases, and as severe in 8 (15 per cent) of the cases.

Neither age, sex, duration of the uremia, nor the degree of azotemia appeared to play a significant rôle in the production of the lesion. The incidence of uremic pericarditis was significantly higher in the group . cases in which the lesion was present. A mild or moderate form of the lesion was present in 40 (20 per cent) of a control series of 200 cases. Although uremia was not listed as a contributory cause of death in any of these cases, azotemia had been present in 10 of the 40 cases. The most common cause of death was intestinal obstruction. Although the cause of the lesion is not clear from this study, it is suggested that dehydration and some as yet unknown metabolic disturbance result in inspissation of secretion and intrinsic obstruction of the pancreatic ductules and acini.

Discussion

(Dr. Tracy B. Mallory, Boston, Mass.) I became very much interested in this lesion which Dr. Baggenstoss has just described and illustrated when I was with the Army in Italy. I find it comparatively uncommon in the material from civilian hospitals whereas I was quite startled by its frequency, which I would estimate at 10 per cent, in the material passing through my laboratory in Italy. Like Dr. Baggenstoss, I observed it in patients dying from renal insufficiency. I noted it, however, with equal frequency and severity in patients with severe infections, particularly peritonitis. The most marked examples which I saw occurred in typhus fever, and all of 24 typhus cases showed the lesion in some degree. We were unable to discover any promising clues pointing toward its etiology, but strongly suspected dehydration. I never found the lesion in any case of sudden death or, in fact, in any individual who had not been severely ill for at least 4 days, usually 6 or more, before death.

(Dr. Theodore Gillman, Chicago, Ill.) The lesion which has just been described

is of great interest to us, because my brother, Dr. Joseph Gillman, and I have been able to produce a very similar reaction in rats by nutritional means. We have also encountered a similar lesion with fibrosis in the pancreas of human subjects who have died with severe malnutrition. In rats the lesion is preceded by, and associated with, severe liver disease, and whether this liver disease plays a direct part remains to be determined. Dorothy Andersen, in her classic work on cystic fibrosis of the pancreas, referred to liver disease being present. Moreover, an examination of her protocols reveals that the liver disease was associated with deposition of pigment granules similar to those which we described in human subjects in South Africa. On the basis of experimental work with rats we have no hesitation in saying the hepatic lesions preceded the development of the lesions in the pancreas. Certainly, in rats, the lesion may progress and the pancreas may atrophy without any fibrosis at all, and all that remains of the pancreas are masses of fatty tissue and isolated islands without any replacement fibrosis. I think that the relationship between the liver and the pancreas in this particular lesion is of importance, especially in view of the fact reported by Dr. Baggenstoss that the presence of uremia and of renal lesions, in particular, may predispose to the lesion in the pancreas. This indicates that the entire body is reacting and the pancreas plays an important rôle in the pathologic process.

(Dr. I. Davidsohn, Chicago, Ill.) Two years ago, I observed lesions in a patient who died of ulcerative colitis. Since that time we had a few cases of the same condition and I found it also in some of the older cases with ulcerative colitis on re-examination. I do not remember that any of the cases had uremia as a significant complication.

(Dr. Mallory) I should like to make one more comment in relation to what Dr. Gillman has said. I had read his paper and was interested in the possibility of dietary deficiency. In the group of cases we studied we had a wide variety of nationalities: Italian civilians, French Moroccan, American, native Indian troops, British, and German prisoners of war, all of whom had widely different backgrounds, but the incidence of the lesion in all these different racial groups was identical.

(Dr. Gillman) May we have some information about the state of the liver in these patients?

(Dr. Baggenstoss) There were no abnormalities noted in the liver in most of the cases. We did have some cases of obstructive jaundice in which uremia developed that revealed the pancreatic lesion; but in most of the cases there were no lesions observed in the liver outside of such incidental ones as chronic passive congestion.

(Dr. Mallory) I think the liver lesions in the cases I saw were coincidental. There were instances of focal necrosis and chronic passive congestion and so on, but nothing constant or specific. In fact I was much interested to observe that we never saw the lesion in association with fatal epidemic hepatitis.

A Histologic Study of Skeletal Muscle in Acute Ischemia.* John W. Harman (by invitation), Madison, Wis.

Abstract. Skeletal muscles of rabbits and white rats, rendered acutely ischemic for periods of from 1 to 96 hours by ligation of vessels and application of tourniquets, were studied histologically and compared with contralateral normal controls. The characteristics of normal rapidly fixed muscles are described as syncytoid structures with vague cross striations and a conspicuousness of undulant longitudinal striations. The nuclei, which are deeply basophilic, contain a fine chromatin network. With ischemia of 2 to 4 hours' duration the fibers are individualized, longitudinal striations disappear, and cross striations become a conspicuous cytologic feature. After longer periods of ischemia abnormal anisotropic disks, called "Bowman's disks" or "conchoidal plates," appear and involve the muscle fibers in

* See also: *Am. J. Path.*, 1947, 23, 551–565.

increasing numbers up to 18 hours of ischemia, at which time they are nearly ubiquitous. They represent true degenerative forms and not artefacts caused by fixation and sectioning technique. Weakness or absence of contractility precedes and accompanies the appearance of these disks, and is correlated with their presence and extent of involvement, so that they serve as an indication of nonviable fibers and represent a morphologic manifestation of cell death in skeletal muscle.

NODULAR INFLAMMATORY AND DEGENERATIVE LESIONS IN MUSCLES FROM 450 AUTOPSIES. B. J. Clawson, J. F. Noble, and (by invitation) N. H. Lufkin, Minneapolis, Minn.

Abstract. Seven muscles (the pectoral, sternomastoid, deltoid, diaphragm, intercostal, psoas, and sacrospinalis) were collected from each of 450 autopsies and studied for evidences of inflammatory and degenerative lesions.

The Inflammatory Lesions. These lesions consisted primarily of infiltration of lymphocytes and plasma cells between the muscles. They were most common in the diaphragm and intercostals and were present in various degrees in one or more muscles in 118 cases (26.2 per cent). These inflammatory lesions were most commonly found in cases of acute rheumatic endocarditis, rheumatoid arthritis, and in older persons with various diseases. The sexes were about equally involved.

The Degenerative Lesions. The degenerative lesions consisted of atrophy, sarcoplasmic changes (Zenker's degeneration and necrosis), and nuclear changes. Atrophy was most common in the diaphragm and sacrospinalis, and sarcoplasmic changes in the diaphragm. Atrophy in different degrees was noted in 191 cases (42.4 per cent) and sarcoplasmic changes in 152 cases (33.8 per cent). Nuclear changes consisted of irregularities in number, shape, and size, and of hyperchromatic staining qualities. These nuclear changes were most common in the deltoid, diaphragm, intercostal, and sacrospinalis. These changes were noted in one or more muscles in 158 cases (35.1 per cent).

One or more of the lesions (myositis, atrophy, sarcoplasmic or nuclear changes) was present in 293 of the 450 cases (65.1 per cent). On a morphologic basis it is doubtful whether any of the muscular lesions can be considered as a specific reaction to the infectious agent of either acute rheumatic or rheumatoid arthritis. These lesions may possibly be a part of the rheumatic and rheumatoid state.

Discussion

(Dr. Virgil H. Cornell, Washington, D.C.) I would like to ask if Dr. Clawson can report on the incidence of trichinosis in the diaphragm.

(Dr. Clawson) Trichinosis was commonly found. It was not included in these plates. Some of these inflammatory lesions possibly may have been on the edge of a trichinosis lesion. I did not work out the incidence. It was relatively high.

(Dr. Cornell) The high percentage in the diaphragm was what struck my eye.

READ BY TITLE

PATHOLOGY OF TRENCH FOOT. Matthew Block (by invitation), Chicago, Ill.

Abstract: Not received.

YELLOW FEVER IN THE AFRICAN. John C. Bugher and (by invitation) Richard G. Hahn, New York, N.Y.

Abstract: Not received.

THE NONESSENTIALITY OF ARGININE FOR SPERMATOGENESIS IN THE ALBINO RAT. Paul R. Cannon and (by invitation) C. Harold Steffee, Robert L. Woodridge, Laurence E. Frazier, and Norman L. Jennings, Chicago, Ill.

Abstract: Not received.

HEMOGLOBIN CRYSTALS, CASTS, AND GLOBULES IN THE RENAL TUBULES OF GUINEA-PIGS FOLLOWING CHEMICAL HEMOLYSIS.* Robert C. Dunn and (by invitation) Stewart H. Webster, Bethesda, Md.

Abstract. An experimental investigation was undertaken of the results in the kidneys of guinea-pigs following hemolysis due to exposure to stibine (SbH_3). Stibine resembles arsine (AsH_3) in its action, but hitherto has been studied very little from the biologic viewpoint. The hemoglobin deposits in the kidney (casts, crystals, and globules) were described in relation to the time elapsing after exposure to stibine. Other changes in the kidney were noted: tubular dilatation, coagulation, necrosis, cellular casts, fat, glomerular changes, and hemosiderosis. The finding and identification of hemoglobin crystals in the renal tubules we believe will be of particular interest. This study is an example of the combined application of physiology, crystallography, and biochemistry in experimental pathology.

THE ALARM REACTION AS A MEASURE OF TOXICITY OF GOITROGENIC COMPOUNDS. William E. Ehrich and (by invitation) Joseph Seifter, Philadelphia, Pa.

Abstract: Not received.

SOME APPLICATIONS OF THE FREEZING-DRYING METHOD FOR MORPHOLOGIC PROBLEMS. Isidore Gersh (by invitation), Chicago, Ill.

Abstract. The principles of the freezing-drying method of fixation make it particularly useful in certain kinds of purely morphologic problems. Several designs (by Gersh, Scott, Sjöstrand, and others) are available for easy application in investigative laboratories. Preservation of substances affected during fixation by water or by enzymes is very satisfactory. Accordingly, the method is particularly suitable for the preservation of inorganic components, glycogen, mucigen granules, edematous tissue, and thyroid colloid. Gas bubbles appearing in blood and/or tissues after decompression are also well preserved. In these instances and in others, the structure after fixation by freezing and drying may be freer of artifact, and may reproduce more closely the living appearance than after more conventional methods of fixation.

EXPERIMENTAL AND SPONTANEOUS BRUCELLOTIC OSTEOMYELITIS OF THE ANIMAL. Leo Lowbeer (by invitation), Tulsa, Okla.

Abstract. At the preceding meeting of this Association, I reported a case of human brucellotic osteomyelitis of the ilium and scapula from which *Brucella suis* was grown in pure culture. Because little is known about the cellular changes of brucellotic osteomyelitis, an attempt was made to reproduce it by inoculation of guinea-pigs with our human strain of *Br. suis*. In addition, material from spontaneous brucellotic osteomyelitis of the hog was obtained through the courtesy of the United States Department of Agriculture.

Of 7 inoculated guinea-pigs, osteomyelitis and periostitis were produced in 2. Both animals were killed 7 weeks after inoculation. One had bilateral exophthalmos caused by retrobulbar abscesses from which *Br. suis* was cultured. Both had pseudo-suppurative orchitis and epididymitis, and typical brucellotic granulomas and pseudo-abscesses in the liver and other organs, all with positive cultures. One animal showed fusiform enlargement of many ribs caused by periosteal osteophytes. There was also osteoclastic absorption of the cortex from a round-cell infiltrated periosteum with fragmentation and formation of sequestra. The cortex was also melting away from the bone marrow side through an apparently humoral process. The marrow showed necrosis and mononuclear cell infiltration. In areas of cortex destruction there was extensive formation of endosteal and periosteal osteophytes and chondrophytes.

* This article will appear in a subsequent issue of *The American Journal of Pathology*.

increasing numbers up to 18 hours of ischemia, at which time they are nearly ubiquitous. They represent true degenerative forms and not artefacts caused by fixation and sectioning technique. Weakness or absence of contractility precedes and accompanies the appearance of these disks, and is correlated with their presence and extent of involvement, so that they serve as an indication of nonviable fibers and represent a morphologic manifestation of cell death in skeletal muscle.

NODULAR INFLAMMATORY AND DEGENERATIVE LESIONS IN MUSCLES FROM 450 AUTOPSIES. B. J. Clawson, J. F. Noble, and (by invitation) N. H. Lufkin, Minneapolis, Minn.

Abstract. Seven muscles (the pectoral, sternomastoid, deltoid, diaphragm, intercostal, psoas, and sacrospinalis) were collected from each of 450 autopsies and studied for evidences of inflammatory and degenerative lesions.

The Inflammatory Lesions. These lesions consisted primarily of infiltration of lymphocytes and plasma cells between the muscles. They were most common in the diaphragm and intercostals and were present in various degrees in one or more muscles in 118 cases (26.2 per cent). These inflammatory lesions were most commonly found in cases of acute rheumatic endocarditis, rheumatoid arthritis, and in older persons with various diseases. The sexes were about equally involved.

The Degenerative Lesions. The degenerative lesions consisted of atrophy, sarcoplasmic changes (Zenker's degeneration and necrosis), and nuclear changes. Atrophy was most common in the diaphragm and sacrospinalis, and sarcoplasmic changes in the diaphragm. Atrophy in different degrees was noted in 191 cases (42.4 per cent) and sarcoplasmic changes in 152 cases (33.8 per cent). Nuclear changes consisted of irregularities in number, shape, and size, and of hyperchromatic staining qualities. These nuclear changes were most common in the deltoid, diaphragm, intercostal, and sacrospinalis. These changes were noted in one or more muscles in 158 cases (35.1 per cent).

One or more of the lesions (myositis, atrophy, sarcoplasmic or nuclear changes) was present in 293 of the 450 cases (65.1 per cent). On a morphologic basis it is doubtful whether any of the muscular lesions can be considered as a specific reaction to the infectious agent of either acute rheumatic or rheumatoid arthritis. These lesions may possibly be a part of the rheumatic and rheumatoid state.

Discussion

(Dr. Virgil H. Cornell, Washington, D.C.) I would like to ask if Dr. Clawson can report on the incidence of trichinosis in the diaphragm.

(Dr. Clawson) Trichinosis was commonly found. It was not included in these plates. Some of these inflammatory lesions possibly may have been on the edge of a trichinosis lesion. I did not work out the incidence. It was relatively high.

(Dr. Cornell) The high percentage in the diaphragm was what struck my eye.

READ BY TITLE

PATHOLOGY OF TRENCH FOOT. Matthew Block (by invitation), Chicago, Ill.

Abstract: Not received.

YELLOW FEVER IN THE AFRICAN. John C. Bugher and (by invitation) Richard G. Hahn, New York, N.Y.

Abstract: Not received.

THE NONESSENTIALITY OF ARGININE FOR SPERMATOGENESIS IN THE ALBINO RAT. Paul R. Cannon and (by invitation) C. Harold Steffee, Robert L. Woodridge, Laurence E. Frazier, and Norman L. Jennings, Chicago, Ill.

Abstract: Not received.

HEMOGLOBIN CRYSTALS, CASTS, AND GLOBULES IN THE RENAL TUBULES OF GUINEA-PIGS FOLLOWING CHEMICAL HEMOLYSIS.* Robert C. Dunn and (by invitation) Stewart H. Webster, Bethesda, Md.

Abstract. An experimental investigation was undertaken of the results in the kidneys of guinea-pigs following hemolysis due to exposure to stibine (SbH_3). Stibine resembles arsine (AsH_3) in its action, but hitherto has been studied very little from the biologic viewpoint. The hemoglobin deposits in the kidney (casts, crystals, and globules) were described in relation to the time elapsing after exposure to stibine. Other changes in the kidney were noted: tubular dilatation, coagulation, necrosis, cellular casts, fat, glomerular changes, and hemosiderosis. The finding and identification of hemoglobin crystals in the renal tubules we believe will be of particular interest. This study is an example of the combined application of physiology, crystallography, and biochemistry in experimental pathology.

THE ALARM REACTION AS A MEASURE OF TOXICITY OF GOITROGENIC COMPOUNDS. William E. Ehrich and (by invitation) Joseph Seifter, Philadelphia, Pa.

Abstract: Not received.

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The other animal started limping shortly before it was sacrificed. Roentgenogram showed a destructive lesion in the epiphysis of the distal left radius. Microscopically, there was replacement of the bone marrow by partly necrotic mononuclear cell granulomas and destruction of cancellous bone; osteoclastic absorption of bony spicules took place in the neighborhood. Multiple similar areas were found in other bones.

Three cases of spontaneous brucellotic spondylitis and one of osteomyelitis of the radius and ulna of hogs with positive cultures of *Br. suis* also were studied. The lesions all resembled those presented by Feldman and Olsen in 1933.

In conclusion, brucellotic osteomyelitis, spontaneous as well as experimental, causes mononuclear cell infiltration and necrosis of the bone marrow, occasionally with the formation of pseudo-abscesses, early osteoclastic absorption of bone through caries sicca and humoral destruction of bone, and very extensive and early peripheral production of new bone. This leads to the formation of a central cavity filled with necrotic, often calcified granulation tissue and sequestra, and surrounded by a fibrotic capsule and new formed bone. The relatively few roentgenologic and clinical studies of human brucellotic osteomyelitis indicate that the process in man is similar to that in the animal.

CYTOLOGY AND INCIDENCE OF INTRANUCLEAR INCLUSIONS IN THE ISLANDS OF LANGERHANS OF THE CHICKEN.* Alfred M. Lucas (by invitation), East Lansing, Mich.

Abstract: Not received.

A SURVEY OF 52 CASES OF ACUTE NECROSIS OF THE LIVER. J. S. McCartney, Minneapolis, Minn.

Abstract: Not received.

RELATIONSHIP OF DEGREE AND DURATION OF HYPERTHERMIA TO THE NATURE OF THE CUTANEOUS INJURY. A. R. Moritz and (by invitation) F. C. Henriques, Jr., Boston, Mass.

Abstract. Predictable reactions of porcine and human skin to thermal exposures were obtained by appropriate regulation of the surface temperature and the duration of the hyperthermic episode. The occurrence of latent and morphologically unrecognizable thermal injury was disclosed by the cumulative effects of repeated subthreshold exposures. The sequence of events during exposure at temperatures of different intensity was studied with particular attention to the occurrence of a type of tissue denaturation that rendered it resistant to lysis and organization.

METASTATIC CALCIFICATION PRODUCED IN DOGS BY HYPERVITAMINOSIS D AND HALIPHAGIA.* R. M. Mulligan, Denver, Colo.

Abstract. Seven dogs receiving vitamin D (as ertron) and sal hepatica (containing the chloride, sulfate, phosphate, and bicarbonate salts of sodium) and 5 given ertron alone showed weight loss, inappetence, hypodypsia, and apathy, which 2 dogs on sal hepatica alone and 2 control dogs did not exhibit. The dogs in each of the 4 groups displayed characteristic stools. All dogs receiving ertron revealed varying calcification in the left atrium, aorta, lungs, stomach, and kidneys, and various degrees of atrophy of the parathyroid glands, prostate, testes, and lymphoid and fat tissues; some of them showed hypoplasia and serous fat atrophy of the bone marrow.

HEMOLYTIC ANEMIA AND DUODENAL ATRESIA IN ONE OF TWINS IN A CASE OF HETEROSPECIFIC PREGNANCY WITH ISO-IMMUNIZATION OF THE MOTHER. Silik H. Polayes, Brooklyn, N.Y.

Abstract: Not received.

* This article will appear in a subsequent issue of *The American Journal of Pathology.*

DIASTASIS AND DIASTATIC PERFORATION OF THE GASTROINTESTINAL TRACT. A CLINICAL, PATHOLOGIC, AND EXPERIMENTAL STUDY. Jacob M. Ravid, New York, N.Y.

Abstract. Diastasis and diastatic perforation of the gastrointestinal tract is a clinicopathologic entity, first described by Heschel in 1880, which has not received due recognition by either clinicians or pathologists. It is a distention, partial tearing, or perforation of a nondiseased bowel, which takes place as a secondary complication in the course of an intrinsic or extrinsic stenosing disease of the bowel situated distally to the site of distention or perforation. It is most common in cancer of the colon, but may occur also with congenital disease of the gastrointestinal tract, volvulus, and various other intrinsic or extrinsic obstructive diseases of the intestines. The clinical picture is that of chronic intestinal obstruction with sudden transition to "an acute abdomen." The competence or incompetence of Bauhin's valve plays an important rôle in this syndrome. Experimental work done in this connection furnishes interesting data with regard to the "tensile" strength of the various portions of the gastrointestinal tract, which can be correlated with the anatomic findings in the observed clinical cases.

THE INFECTIVITY OF SYPHILITIC MOUSE ORGANS. Paul D. Rosahn and (by invitation) Boris Gueft, New Britain, Conn.

Abstract. Groups of mice were inoculated by different routes with rabbit chancre emulsions. At variable intervals following inoculation different mouse organs were subinoculated into rabbits which were observed for the development of darkfield-positive lesions. Spleen and lymph nodes were regularly infectious at 10, 20, 30, 45, and 90 days following mouse inoculation. The frequency of successful transfers to rabbits, when infected mouse skin was employed, increased progressively to almost 100 per cent at 90 days after inoculation. Mouse brain, however, was noninfectious at 10 and 20 days, and at 90 days after inoculation was capable of producing lesions in rabbits in only about half of the trials. The findings varied somewhat, depending on the method of inoculation.

THE CONTAGIOUSNESS OF COCCIDIOIDOMYCOSIS. AN EXPERIMENTAL STUDY. Sol Roy Rosenthal, Chicago, Ill.

Abstract. The spherules of *Coccidioides immitis* remain viable under certain conditions and do not produce hyphae and chlamydospores in exudates from human sources for at least 110 days (experiments still in progress). By instilling sputum or exudates from human or animal sources into the bronchi of guinea-pigs and propelling them by air pressure into the finer ramifications of the bronchioles and alveoli, it is possible to produce coccidioidomycosis infections in 100 per cent of the animals. The lesions localize for the most part in the upper portions of the lung, are single or multiple, have a lymph node component, and do not generalize; thus simulating the human infection. These experiments show that spherules (or sporangia) can be infective through the respiratory route from man to animal, and from animal to animal. It is concluded that until proved otherwise, active human cases of primary progressive coccidioidomycosis should be considered contagious.

GASTRIC CANCER: A COMPARISON OF THE GROSS AND MICROSCOPIC DIFFERENCES BETWEEN SHORT TERM AND FIVE-YEAR SURVIVORS AFTER GASTRECTOMY. Paul E. Steiner and (by invitation) S. N. Maimon, Walter L. Palmer, and Joseph B. Kirsner, Chicago, Ill.

Abstract. Thirty patients who survived for 5 years or more after gastric resection for cancer were compared with 30 who died of local recurrence or metastases within 1 year after a similar operation. Attempts were made to find points of prognostic significance between the two groups by studying the clinical data, and the gross and microscopic appearances of the tumors. Differences in the clinical

data were not striking, but the cancers in the 5-year survivors were grossly more sharply circumscribed as indicated by a greater percentage of the lower types by the Borrmann method. Three histologic differences were found between the two groups. In order of importance they were: (a) sharp circumscription with growth of the tumor through the gastric wall *en bloc*, seen in most of the 5-year but in few of the short-term survivors; (b) retrogressive changes consisting of atrophy, nuclear pyknosis, and degeneration, found in a few 5-year survivors (these changes resemble those present in some prostatic carcinomas following orchiectomy); (c) two special histologic types of cancer: one an undifferentiated, medullary small-celled carcinoma with leukocyte-rich stroma; the other an undifferentiated round cell sarcoma or carcinoma. Presence of metastases at the time of operation was not incompatible with 5-year survival. Biologically, the long-term survivors were not a homogeneous group. Survival for 5 years was explained by complete removal of tumor cells in some cases and by slow rate of local recurrence or metastasis in others. The biologic resistance to rapid growth was anatomically manifest by the three histologic features mentioned, which can be used to advantage in forecasting prognosis in gastric cancer.

LOCALIZATION OF THE KIDNEY DAMAGE INDUCED BY *dl*-SERINE IN THE RAT. PHOSPHATASE ACTIVITY, INFLUENCE OF AGE, SEX, TIME, AND DOSE. PROTECTIVE ACTION OF VARIOUS AMINO ACIDS AND SOME OTHER COMPOUNDS. Max Wachstein, Middletown, N.Y.

Abstract. The findings of Fishman, Artom, and Morehead concerning the nephrotoxic action of *dl*-serine in rats were fully confirmed. Not only male animals weighing 100 gm. but also weanlings and adult rats are susceptible. In female rats, likewise, *dl*-serine exerts a nephrotoxic action, although less regularly.

The minimal injurious dose for male rats weighing 100 gm. on a synthetic diet poor in the B vitamins and deficient in protein was found to be 10 mg., while 20 to 30 mg. led in most animals to extensive necrosis. As early as 30 minutes after intravenous injection of *dl*-serine, distinct kidney lesions were observed in some instances. The renal damage was found to be localized in the terminal portions of the proximal convoluted tubules. After the introduction of *dl*-serine, phosphatase activity was still found 24 hours later in the cells of necrotic tubules.

The severe necrotizing nephrosis induced by *dl*-serine can be favorably influenced by several amino acids and related compounds. *dl*-Methionine and glutathione exert a considerable protective influence. This is probably not due to SH-groups, since cysteine and thioglycolic acid have only little, and 2·3-dithiopropanol (BAL) has no, beneficial effect. *dl*-α-Alanine, glycine, *dl*-threonine, glycolic acid, butyric, and pyruvic acids have a very considerable protective influence. *l* (+) Histidine monohydrochloride and lactic acid afford appreciable, *l* (+) arginine monohydrochloride and *dl*-valine moderate, protection. Some influence was seen from *l* (+) glutamic acid, while glucose, sodium acetate, and sodium chloride were without any effect. It is assumed that the beneficial effect of the various amino acids and other substances against the nephrotoxic action of *dl*-serine is due to their competitive suppression of tubular reabsorption of the injurious *d*-isomer.

HEPATOMA REMOVED WITH, TO DATE, A FIVE MONTH CLINICAL RECOVERY. J. W. Williams and (by invitation) C. C. Woods, Bay Pines, Fla.

Abstract. A 64-year-old white male began to have dizzy spells and peristaltic pain in the upper abdomen, worse at night, aggravated by food, and followed by nausea 4 months ago. At operation, a single tumor mass, replacing the left lobe of the liver and the size of a cocoanut, was removed surgically. On section the tumor was brownish red and of scrambled egg consistency. Microscopically, it showed areas ranging in appearance from leiomyosarcoma, and angiosarcoma to hepatoma. The patient gained weight, and now, 5 months after operation, is clinically well.